

UNITED STATES DISTRICT COURT  
District of Columbia

GLAXOSMITHKLINE BIOLOGICALS, S.A.

SUMMONS IN A CIVIL CASE

V.

HON. JOHN J. DOLL  
Acting Under Secretary of Commerce  
for Intellectual Property and  
Acting Director of the U.S. Patent  
and Trademark Office

CASE NUMBER:

Case: 1:09-cv-00398  
Assigned To : Leon, Richard J.  
Assign. Date : 2/27/2009  
Description: Admin. Agency Review

TO: (Name and address of Defendant)

Hon. John J. Doll  
c/o Office of General Counsel, U.S. Patent and Trademark Office  
P.O. Box 15667, Arlington, VA 22215  
Madison Building East, 600 Dulany Street, Alexandria, VA 22314

**YOU ARE HEREBY SUMMONED** and required to serve on PLAINTIFF'S ATTORNEY (name and address)

Bruce R. Genderson  
Williams & Connolly LLP  
725 Twelfth Street, N.W.  
Washington, DC 20005

an answer to the complaint which is served on you with this summons, within 60 days after service of this summons on you, exclusive of the day of service. If you fail to do so, judgment by default will be taken against you for the relief demanded in the complaint. Any answer that you serve on the parties to this action must be filed with the Clerk of this Court within a reasonable period of time after service.

NANCY M. MAYER-WHITTINGTON

FEB 27 2009

CLERK

DATE

(By) DEPUTY CLERK

## RETURN OF SERVICE

Service of the Summons and complaint was made by me <sup>(1)</sup>	DATE <u>MARCH 4, 2009</u>
NAME OF SERVER (PRINT) <u>DIANE YAGER</u>	TITLE <u>LEGAL ASSISTANT</u>

Check one box below to indicate appropriate method of service

☐ Served personally upon the defendant. Place where served: \_\_\_\_\_☐ Left copies thereof at the defendant's dwelling house or usual place of abode with a person of suitable age and discretion then residing therein.

Name of person with whom the summons and complaint were left: \_\_\_\_\_


☐ Returned unexecuted: \_\_\_\_\_☒ Other (specify): CERTIFIED MAIL, RETURN RECEIPT

## STATEMENT OF SERVICE FEES

TRAVEL	SERVICES	TOTAL
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## DECLARATION OF SERVER

I declare under penalty of perjury under the laws of the United States of America that the foregoing information contained in the Return of Service and Statement of Service Fees is true and correct.

Executed on MARCH 4, 2009  
Date  
Signature of Server725 12TH ST. NW, WASHINGTON, DC 20005  
Address of Server

(1) As to who may serve a summons see Rule 4 of the Federal Rules of Civil Procedure.

IN THE UNITED STATES DISTRICT COURT  
FOR THE DISTRICT OF COLUMBIA

GLAXOSMITHKLINE  
BIOLOGICALS, S.A.  
Rue de l'Institut 89  
B-1330 Rixensart, Belgium

Plaintiff,

v.

HON. JOHN J. DOLL  
Acting Under Secretary of Commerce for  
Intellectual Property and Acting Director of  
the United States Patent and Trademark  
Office  
U.S. Patent and Trademark Office  
Madison Building  
600 Dulany Street,  
Alexandria, VA 22314  
Office of General Counsel  
P. O. Box 15667  
Arlington, VA 22215

Defendant.

No.

**Case: 1:09-cv-00398**

**Assigned To : Leon, Richard J.**

**Assign. Date : 2/27/2009**

**Description: Admin. Agency Review**

Plaintiff GlaxoSmithKline Biologicals, S.A., for their complaint against defendant  
the Honorable John J. Doll, state as follows:

**COMPLAINT**

1. This is an action by the owner of United States Patent No. 7,419,824 (the  
"824 patent") seeking review of a final determination by the United States Patent and  
Trademark Office ("PTO") making inaccurate and erroneous patent term adjustment  
calculations for this patent. Plaintiff is seeking a judgment, pursuant to 35 U.S.C.  
§ 154(b)(4)(A), that the patent term adjustment of 458 days calculated by the PTO for the  
'824 patent be changed to 964 days.

2. This action arises under 35 U.S.C. § 154 and the Administrative Procedure  
Act, 5 U.S.C. §§ 701-706.

### **THE PARTIES**

3. Plaintiff GlaxoSmithKline Biologicals, S.A. is a Belgian corporation, having a principal place of business at Rue de l'Institut 89, B-1330 Rixensart, Belgium.

4. Defendant John J. Doll is the Acting Under Secretary of Commerce for Intellectual Property and Acting Director of the PTO, acting and sued in his official capacity. The Acting Director is the head of the PTO and is responsible for superintending or performing all duties required by law with respect to the granting and issuing of patents, and is designated by statute as the official responsible for determining the period of patent term adjustments under 35 U.S.C. § 154.

### **JURISDICTION AND VENUE**

5. This Court has jurisdiction over this action and is authorized to issue the relief sought pursuant to 28 U.S.C. §§ 1331, 1338(a) and 1361, 35 U.S.C. § 154(b)(4)(A), and 5 U.S.C. §§ 701-706.

6. Venue is proper in this district pursuant to 35 U.S.C. § 154(b)(4)(A).

7. This Complaint is being timely filed in accordance with 35 U.S.C. § 154(b)(4)(A).

### **BACKGROUND**

8. The '824 patent issued to Joelle Thormard on September 2, 2008 based on patent application number 10/742,345 (the "'345 application'"), which was filed on December 19, 2003. The '824 patent is attached as Exhibit A.

9. Plaintiff GlaxoSmithKline Biologicals, S.A. is the assignee of the '824 patent, as evidenced by the records recorded in the PTO, and is the real party in interest in this case.

10. Section 154 of 35 U.S.C. requires that the Director of the PTO grant a patent term adjustment in accordance with the provisions of section 154(b). Specifically, 35 U.S.C. § 154(b)(3)(D) states that “[t]he Director shall proceed to grant the patent after completion of the Director’s determination of a patent term adjustment under the procedures established under this subsection, notwithstanding any appeal taken by the applicant of such determination.”

11. In calculating the patent term adjustment, the Director must take into account PTO delays under 35 U.S.C. § 154(b)(1), any overlapping periods of PTO delays under 35 U.S.C. § 154(b)(2)(A), and any applicant delays under 35 U.S.C. § 154(b)(2)(C).

12. The error in the PTO’s patent term adjustment calculation is detailed in this Court’s recent order in *Wyeth v. Dudas*, 580 F. Supp. 2d. 138 (D.D.C. 2008), *appeal docketed*, No. 08-5502 (Fed. Cir. Dec. 24, 2008), which granted summary judgment against the PTO and held that the PTO’s patent term adjustment calculation methodology was erroneous as a matter of law and inconsistent with 35 U.S.C. § 154. The *Wyeth v. Dudas* opinion is attached as Exhibit B.

13. The correct patent term adjustment methodology identified in *Wyeth v. Dudas* governs the PTO’s calculation of patent term adjustment for Plaintiff’s ‘824 patent.

14. Under 35 U.S.C. § 154(b)(4)(A), “[a]n applicant dissatisfied with a determination made by the Director under paragraph (3) shall have remedy by a civil action against the Director filed in the United States District Court for the District of

Columbia within 180 days after the grant of the patent. Chapter 7 of title 5 shall apply to such action.”

15. The PTO issued the ‘824 patent on September 2, 2008 with an incorrect patent term adjustment.

16. The September 2, 2008 patent issuance with the incorrect patent term adjustment is therefore a final decision of the PTO and directly appealable to this Court within 180 days of September 2, 2008 pursuant to 35 U.S.C. § 154(b)(4)(A).

### COUNT I

17. The allegations of paragraphs 1-16 are incorporated in this count as if fully set forth herein.

18. The ‘345 application was filed on December 19, 2003, and issued as the ‘824 patent on September 2, 2008.

19. Under 35 U.S.C. § 154(b)(1)(A), a period of 506 days of PTO delay is attributable to the failure by the PTO to mail an action under 35 U.S.C. § 132 not later than 14 months after the actual filing date of the application (*i.e.*, by February 19, 2005).

20. Under 35 U.S.C. § 154(b)(1)(B), a period of 623 days of PTO delay is attributable to the number of days the issue date of the ‘824 patent exceeds three years from the filing date of the application (“Three Year Delay”).

21. 35 U.S.C. § 154(b)(2)(A) states that “[t]o the extent that periods of delay attributable to grounds specified in paragraph [154(b)(1)] overlap, the period of any adjustment granted under this subsection shall not exceed the actual number of days the issuance of the patent was delayed.” Of the delays attributable to the PTO for the ‘824 patent, there is no period of overlap.

22. Under the PTO's interpretation of 35 U.S.C. § 154, all patent term adjustments accrued under 35 U.S.C. § 154(b)(1)(A) and 35 U.S.C. § 154(b)(1)(B) inherently overlap and, thus, it has been the PTO's position that a patent holder is only eligible for the larger of these two amounts. Thus, the PTO erroneously calculated the total period of PTO delay for the '824 patent as 623 days.

23. Under the interpretation of this Court in *Wyeth v. Dudas* (attached as Exhibit B), both the PTO delay under 35 U.S.C. § 154(b)(1)(A) of 506 days and the Three Year Delay under 35 U.S.C. § 154(b)(1)(B) of 623 days each qualify for patent term adjustment. Thus, the total period of the PTO delay for the '824 patent is 1129 days.

24. Under 35 U.S.C. § 154(b)(2)(C), the total period of the PTO delay is reduced by the period of applicant delay. The PTO calculated the total applicant prosecution delay of 165 days under 35 U.S.C. § 154(b)(2)(C).

25. Accordingly, the correct patent term adjustment under 35 U.S.C. § 154(b)(1) and (2) is 964 days, which is the difference between the total period of the PTO delay (1129 days) and the period of applicant delay (165 days).

26. The PTO erroneously limited the patent term adjustment for the '824 patent to 458 days (a PTO delay of 623 days minus the applicant delay of 165 days), as shown on the face of the patent. (See Exhibit A).

27. The Defendant's denial of the full patent term adjustment of 964 days under 35 U.S.C. § 154(b) for the '824 patent is arbitrary, capricious, an abuse of discretion, or otherwise not in accordance with law and in excess of statutory jurisdiction, authority or limitation.


WHEREFORE, Plaintiff respectfully prays that this Court:

28. Issue an Order changing the period of patent term adjustment for the '824 patent term from 458 days to 964 days and requiring Defendant to alter the term of the '824 patent to reflect the 964-day patent term adjustment due the '824 patent.

29. Grant such other and further relief as the nature of the case may admit or require and as may be just and equitable.

Dated: February 27, 2009

Respectfully submitted,

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# Exhibit A



US007419824B2

(12) **United States Patent**  
**Thonnard**

(10) **Patent No.:** **US 7,419,824 B2**  
(45) **Date of Patent:** **Sep. 2, 2008**

(54) **BASB006 POLYPEPTIDES FROM *NEISSERIA MENINGITIDIS* AND IMMUNOGENIC COMPOSITIONS THEREOF**

(75) **Inventor:** **Joelle Thonnard, Gembloux (BE)**

(73) **Assignee:** **GlaxoSmithKline Biologicals, S.A., Rixensart (BE)**

(\*) **Notice:** Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 458 days.

(21) **Appl. No.:** **10/742,345**

(22) **Filed:** **Dec. 19, 2003**

(65) **Prior Publication Data**

US 2004/0137530 A1 Jul. 15, 2004

**Related U.S. Application Data**

(62) Division of application No. 09/673,896, filed as application No. PCT/EP99/02766 on Apr. 20, 1999, now Pat. No. 6,696,062.

(30) **Foreign Application Priority Data**

Apr. 24, 1998 (GB) ..... 9808866.9

(51) **Int. Cl.**  
**C07H 21/04** (2006.01)  
**C12N 15/00** (2006.01)

(52) **U.S. Cl.** ..... 435/320.1; 435/252.3; 435/254.11;  
435/257.2; 536/23.7; 514/44

(58) **Field of Classification Search** ..... None  
See application file for complete search history.

(56) **References Cited**

**U.S. PATENT DOCUMENTS**

6,914,131 B1 7/2005 Scarlato et al.

**FOREIGN PATENT DOCUMENTS**

EP	0 301 992 A	2/1989
WO	WO 93/ 06861 A	4/1993
WO	WO 96/05858 A1	2/1996
WO	WO 96/33276 A	10/1996
WO	WO 97/26359 A	7/1997
WO	WO 99/24578	5/1999

**OTHER PUBLICATIONS**

Protein Accession No. P44596 (Nov. 1, 1995) Fleischman, et al. Swiss Protein Database.

Fleischman, R.D., et al. "Whole Genome Random Sequencing and Assembly of *Haemophilus Influenzae* Rd" *Science*, 269:5223 pp. 496-512.

PCT International Search Report from PCT/EP99/02766 dated Jan. 13, 2000.

EP International Preliminary Examination Report from PCT/EP99/02766 dated Aug. 18, 2000.

Third Party Observations from EP 1 071 783 dated Jun. 26, 2001.

*Primary Examiner*—Patricia A Duffy

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(57) **ABSTRACT**

The invention provides BASB006 polypeptides and polynucleotides encoding BASB006 polypeptides and methods for producing such polypeptides by recombinant techniques. Also provided are diagnostic, prophylactic and therapeutic uses.

**20 Claims, 19 Drawing Sheets**

**Figure 1A**

Identity to SeqID No:1 is indicated by a dot, while a dash (-) indicates a nonexistent nucleotide.

Seqid1	: ATGAAAACAACCGACAAACGGACAACCGAAACACACCGCAAAGCCCCGAA	: 50
Seqid3	: .....	: 50
Seqid1	: AACCGGTCGCATCCGCTTCTCGCCTGCTTACTTAGCCATATGCCTGTCGT	: 100
Seqid3	: .....C.....	: 100
Seqid1	: TCGGCATTCTTCCCCAAGCCTGGGCGGGACACACTTATTTTCGGCATCAAC	: 150
Seqid3	: .....	: 150
Seqid1	: TACCAATACTATCGCGACTTTGCCGAAAATAAAGGCAAGTTTGCAGTCGG	: 200
Seqid3	: .....	: 200
Seqid1	: GGCGAAAGATATTGAGGTTTACAACAAAAAAGGGGAGTTGGTCGGCAAAT	: 250
Seqid3	: .....	: 250
Seqid1	: CAATGACAAAAGCCCCGATGATTGATTTTCTGTGGTGTTCGCGTAACGGC	: 300
Seqid3	: .....	: 300
Seqid1	: GTGGCGGCATTGGTGGGCGATCAATATATTGTGAGCGTGGCACATAACGG	: 350
Seqid3	: .....	: 350

Figure 1B

Seqid1	CGGCTATAACAACGTTGATTTTGGTGCGGAGGGAAGCAATCCCGATCAGC	400
Seqid3	.....A.....A.....A.....	400
Seqid1	ACCGTTTTTCTTATCAAATTGTGAAAAGAAATAATTATAAAGCAGGGACT	450
Seqid3	.T.....A.....A.....C.G.....	450
Seqid1	AACGGTCATCCTTATGGTGGCGATTATCATATGCCGCGTTTACATAAAATT	500
Seqid3	..A..C.....	500
Seqid1	TGTAACCGATGCAGAACCTGTTGAAATGACCAGTTATATGGATGGGCGGA	550
Seqid3	...C..A.....	550
Seqid1	AATATATCGATCAAAATAATTACCCTGACCGTGTTCGTATTGGGGCAGGC	600
Seqid3	.....	600
Seqid1	AGGCAATATTGGCGATCTGATGAAGATGAGCCCAATAACCGCGAAAGTTC	650
Seqid3	.....	650
Seqid1	ATATCATATTGCAAGTGCGTATTCTTGGCTCGTTGGTGGCAATACCTTTG	700
Seqid3	.....	700
Seqid1	CACAAAATGGATCAGGTGGTGGCACAGTCAACTTAGGTAGTGAAAAAATT	750
Seqid3	.....	750

Figure 1C

Seqid1 :               760               \*               780               \*               800  
Seqid3 : ..... : 800

Seqid1 :               \*               820               \*               840               \*  
Seqid3 : ..... : 850

Seqid1 :               860               \*               880               \*               900  
Seqid3 : ..... : 900

Seqid1 :               \*               920               \*               940               \*  
Seqid3 : ..... : 950

Seqid1 :               960               \*               980               \*               1000  
Seqid3 : .....G.....T....A... : 1000

Seqid1 :               \*               1020               \*               1040               \*  
Seqid3 : ..G..... : 1050

Seqid1 :               1060               \*               1080               \*               1100  
Seqid3 : ..... : 1100

Seqid1 :               \*               1120               \*               1140               \*  
Seqid3 : ..... : 1150

## Figure 1D

```

      1160      *      1180      *      1200
Seqid1 : GTTATCGACCCAGACTGAATAATGGAGAAAATATTTCCCTTTATTGACGAA : 1200
Seqid3 : ..... : 1200

      *      1220      *      1240      *
Seqid1 : GGAAAAGGCGAATTGATACTTACCAGCAACATCAATCAAGGTGCTGGAGG : 1250
Seqid3 : ..... : 1250

      1260      *      1280      *      1300
Seqid1 : ATTATATTTCCAAGGAGATTTTACGGTCTCGCCTGAAAATAACGAAACGT : 1300
Seqid3 : ..... : 1300

      *      1320      *      1340      *
Seqid1 : GGCAAGGTGCGGGCGTTCATATCAGTGAAGACAGTACCGTTACTTGAAA : 1350
Seqid3 : ..... : 1350

      1360      *      1380      *      1400
Seqid1 : GTAAACGGCGTGGCAAACGACCGCTGTCCAAAATCGGCAAAGGCACGCT : 1400
Seqid3 : ..... : 1400

      *      1420      *      1440      *
Seqid1 : GCACGTTCAAGCCAAAGGGGAAAACCAAGGCTCGATCAGCGTGGGCGACG : 1450
Seqid3 : ..... : 1450

      1460      *      1480      *      1500
Seqid1 : GTAAAGTTATTTTAGATCAACAAGCAGATGAAAATAATAAAAAACAAGCC : 1500
Seqid3 : ...C...G...G...G...C..T..AGGC..... : 1500

      *      1520      *      1540      *
Seqid1 : TTTAGTCAAATCGGCTTGGTCAGCGGCAGGGGTACGGTGCAACTGAATGC : 1550
Seqid3 : ..... : 1550
```

Figure 1E

```

                1560          *          1580          *          1600
Seqid1 : CGATAATCAGTTCAACCCCGACAAACTCTATTTTCGGCTTTTCGCGGCGGAC : 1600
Seqid3 : ..... : 1600

                *          1620          *          1640          *
Seqid1 : GTTTGGATTTGAACGGGCATTTCGTTTCGTTCCACCGTATTCAAATACC : 1650
Seqid3 : .....A..... : 1650

                1660          *          1680          *          1700
Seqid1 : GATGAAGGGGCGATGATTGTCAACCACAATCAAGACAAAGAATCCACCGT : 1700
Seqid3 : ..... : 1700

                *          1720          *          1740          *
Seqid1 : TACCATTACAGGCAATAAAGATATTGCTACAACCGGCAATAACAACAGCT : 1750
Seqid3 : ..... : 1750

                1760          *          1780          *          1800
Seqid1 : TGGATAGCAAAAAAGAAATTGCCTACAACGGTTGGTTTGGCGAGAAAGAT : 1800
Seqid3 : ..... : 1800

                *          1820          *          1840          *
Seqid1 : ACGACCAAAACGAACGGGCGGCTCAACCTTGTTTTACCAGCCCCGCCGAGA : 1850
Seqid3 : ..... : 1850

                1860          *          1880          *          1900
Seqid1 : AGACCGCACCCCTGCTGCTTTCCGGCGGAACAAATTTAAACGGTAACATCA : 1900
Seqid3 : .....C..... : 1900

                *          1920          *          1940          *
Seqid1 : CGCAAACAAACGGCAAACCTGTTTTTCAGCGGCAGACCGACACCGCACGCC : 1950
Seqid3 : .....A..... : 1950
```

Figure 1F

1960                   \*                   1980                   \*                   2000  
Seqid1 : TACAATCATTTAGGAAGCGGGTGGTCAAAAATGGAAGGTATCCCACAAGG : 2000  
Seqid3 : .....AACGA.CAT....GC..AA..G..C..T...GC.. : 2000

                 \*                   2020                   \*                   2040                   \*  
Seqid1 : AGAAATCGTGTGGGACAACGACTGGATCAACCGCACGTTTAAAGCGGAAA : 2050  
Seqid3 : G.....A..... : 2050

2060                   \*                   2080                   \*                   2100  
Seqid1 : ATTTCCATATTCAGGGCGGGCAGGCGGTGATTTCCCGCAATGTTGCCAAA : 2100  
Seqid3 : .C....A..A..A...A.....G..... : 2100

                 \*                   2120                   \*                   2140                   \*  
Seqid1 : GTGGAAGGCGATTGGCATTTGAGCAATCACGCCCAAGCAGTTTTTGGTGT : 2150  
Seqid3 : ..A..... : 2150

2160                   \*                   2180                   \*                   2200  
Seqid1 : CGCACCGCATCAAAGCCACACAATCTGTACACGTTTCGGACTGGACGGGTC : 2200  
Seqid3 : ..... : 2200

                 \*                   2220                   \*                   2240                   \*  
Seqid1 : TGACAAATTGTGTCGAAAAAACCATTACCGACGATAAAGTGATTGCTTCA : 2250  
Seqid3 : ..... : 2250

2260                   \*                   2280                   \*                   2300  
Seqid1 : TTGACTAAGACCGACATCAGCGGCAATGTCAGCCTTGCCGATCACGCTCA : 2300  
Seqid3 : .....GAT..... : 2300

                 \*                   2320                   \*                   2340                   \*  
Seqid1 : TTTAAATCTCACAGGGCTTGCCCACTCAACGGCAATCTTAGTGCAAATG : 2350  
Seqid3 : ..... : 2350



## Figure 1G

Seqid1 : GCGATACACGTTATACAGTCAGCCACAACGCCACCCAAAACGGCGACCTT : 2400  
Seqid3 : .....A.... : 2400

Seqid1 : AGCCTCGTGGGCAATGCCCAAGCAACATTTAATCAAGCCACATTAAACGG : 2450  
Seqid3 : ..... : 2450

Seqid1 : CAACACATCGGCTTCGGGCAATGCTTCATTTAATCTAAGCAACAACGCCG : 2500  
Seqid3 : .....G..C..... : 2500

Seqid1 : TACAAAACGGCAGTCTGACGCTTCCGGCAACGCTAAGGCAAACGTAAGC : 2550  
Seqid3 : ..... : 2550

Seqid1 : CATTCGCACTCAACGGTAATGTCTCCCTAGCCGATAAGGCAGTATTCCA : 2600  
Seqid3 : ..... : 2600

Seqid1 : TTTTGAAAGCAGCCGCTTTACCGGACAAATCAGCGGCAGCAAGGATACGG : 2650  
Seqid3 : .....G..... : 2650

Seqid1 : CATTACACTTAAAAGACAGCGAATGGACGCTGCCGTCAGGCACGGAATTA : 2700  
Seqid3 : ..... : 2700

Seqid1 : GGCAATTTAAACCTTGACAACGCCACCATTACACTCAATTCCGCCTATCG : 2750  
Seqid3 : ..... : 2750

## Figure 1H

```
                2760          *          2780          *          2800
Seqid1 : CCACGATGCGGCGAGGGGCGCAAACCGGCAGTGCACAGATGCGCCGCGC- : 2799
Seqid3 : .....C : 2800

                *          2820          *          2840          *
Seqid1 : -----CGCCGTTTCGCGCCGTTCCCTATTATCCGTTACACCTCCGGCT : 2841
Seqid3 : GCCGTTTCG.....G..AA.. : 2850

                2860          *          2880          *          2900
Seqid1 : TCGGCAGAAATCCCATTTCACACGCTGACGGTAAACGGCAAATTGAACGG : 2891
Seqid3 : ...T.....G..... : 2900

                *          2920          *          2940          *
Seqid1 : TCAGGGAACATTCCGCTTTATGTCGGAACCTCTTCGGCTACCGAAGCGACA : 2941
Seqid3 : .....C..... : 2950

                2960          *          2980          *          3000
Seqid1 : AATTGAAGCTGGCGGAAAGTTCCGAAGGCACCTTACACCTTGGCGGTCAAC : 2991
Seqid3 : ..... : 3000

                *          3020          *          3040          *
Seqid1 : AATACCGGCAACGAACCCGTAAGCCTCGATCAATTGACGGTAGTGAAGG : 3041
Seqid3 : .....T.C.....T..A..... : 3050

                3060          *          3080          *          3100
Seqid1 : GAAAGACAACAAACCGCTGTCCGAAAACCTTAATTTACACCTGCAAAACG : 3091
Seqid3 : A.....T.....T..... : 3100

                *          3120          *          3140          *
Seqid1 : AACACGTCGATGCCGCGCGTGGCGTTACCAACTCATCCGCAAAGACGGC : 3141
Seqid3 : ..... : 3150
```

## Figure 11

3160 \* 3180 \* 3200  
Seqid1 : GAGTTCCGCCTGCATAATCCGGTCAAAGAACAAGAGCTTTCCGACAAACT : 3191  
Seqid3 : ..... : 3200

\* 3220 \* 3240 \*  
Seqid1 : CGGCAAGGCAGAAGCCAAAAACAGGCGGGAAAAGACAACGCGCAAAGCC : 3241  
Seqid3 : .....A..... : 3250

3260 \* 3280 \* 3300  
Seqid1 : TTGACGCGCTGATTGCGGCCGGGCGCGATGCCGTCGAAAAGACAGAAAGC : 3291  
Seqid3 : ..... : 3300

\* 3320 \* 3340 \*  
Seqid1 : GTTGCCGAACCGGCCCGGCAGGCAGGCGGGGAAAATGTCGGCATTATGCA : 3341  
Seqid3 : ..... : 3350

3360 \* 3380 \* 3400  
Seqid1 : GGCGGAGGAAGAGAAAAACGGGTGCAGGCGGATAAAGACACCGCCTTGG : 3391  
Seqid3 : ..... : 3400

\* 3420 \* 3440 \*  
Seqid1 : CGAAACAGCGCGAAGGGAAAACCCGGCCGGCTACCACCGCCTTCCCCCGC : 3441  
Seqid3 : .....C.G..... : 3450

3460 \* 3480 \* 3500  
Seqid1 : GCGCGCCGCGCCCGCGGATTTGCCGCAACCGCAGCCCCAACCGCAACC : 3491  
Seqid3 : .....T...A.....G... : 3500

\* 3520 \* 3540 \*  
Seqid1 : CCAACCGCAGCGCGACCTGATCAGCCGTTATGCCAATAGCGGTTTGAGTG : 3541  
Seqid3 : ..... : 3550

Figure 1J

3560 \* 3580 \* 3600  
Seqid1 : AATTTTCCGCCACGCTCAACAGCGTTTTCGCCGTACAGGACGAATTAGAC : 3591  
Seqid3 : ..... : 3600

\* 3620 \* 3640 \*  
Seqid1 : CGCGTATTTGCCGAAGACCGCCGCAACGCCGTTTGGACAAGCGGCATCCG : 3641  
Seqid3 : .....A..... : 3650

3660 \* 3680 \* 3700  
Seqid1 : GGACACCAAACACTACCGTTCGCAAGATTTCCGCGCCTACCGCCAACAAA : 3691  
Seqid3 : ..... : 3700

\* 3720 \* 3740 \*  
Seqid1 : CCGACCTGCGCCAAATCGGTATGCAGAAAAACCTCGGCAGCGGGCGCGTC : 3741  
Seqid3 : ..... : 3750

3760 \* 3780 \* 3800  
Seqid1 : GGCATCCTGTTTTTCGCACAACCGGACCGAAAAACCTTCGACGACGGCAT : 3791  
Seqid3 : ..... : 3800

\* 3820 \* 3840 \*  
Seqid1 : CGGCAACTCGGCACGGCTTGCCCCACGGCGCCGTTTTCGGGCAATACGGCA : 3841  
Seqid3 : ..... : 3850

3860 \* 3880 \* 3900  
Seqid1 : TCGGCAGGTTTCGACATCGGCATCAGCACGGGCGCGGGTTTTAGCAGCGGC : 3891  
Seqid3 : ...A.....T.....G..... : 3900

\* 3920 \* 3940 \*  
Seqid1 : AGTCTTTTCAGACGACATCGGAAGCAAAATCCGCCCGCGTGCTGCATTA : 3941  
Seqid3 : ..C.....G.....G..... : 3950

## Figure 1K

```

          3960          *          3980          *          4000
Seqid1 : CGGCATTAGGCACGATACCGCGCCGGTTTCGGCGGATTTCGGCATCGAAC : 3991
Seqid3 : ..... : 4000

          *          4020          *          4040          *
Seqid1 : CGCACATCGGCGCAACGCGCTATTTCGTCCAAAAAGCGGATTACCGCTAC : 4041
Seqid3 : ..... : 4050

          4060          *          4080          *          4100
Seqid1 : GAAAACGTCAATATCGCCACCCCGGCCTTGCGTTCAACCGCTACCGCGC : 4091
Seqid3 : .....A..... : 4100

          *          4120          *          4140          *
Seqid1 : GGCATTAAGGCAGATTATTCATTCAAACCGGCGCAACACATTTCCATCA : 4141
Seqid3 : ..... : 4150

          4160          *          4180          *          4200
Seqid1 : CGCCTTATTTGAGCCTGTCTTATACCGATGCCGCTTCGGGCAAAGTCCGA : 4191
Seqid3 : ..... : 4200

          *          4220          *          4240          *
Seqid1 : ACGCGGTCAATACCGCCGTATTGGCTCAGGATTTTCGGCAAACCCGCAG : 4241
Seqid3 : ..A..... : 4250

          4260          *          4280          *          4300
Seqid1 : TCGGGAATGGGGCGTAAACGCCGAAATCAAAGGTTTCACGCTGTCCCTCC : 4291
Seqid3 : .....C..... : 4300

          *          4320          *          4340          *
Seqid1 : ACGCTGCCGCCGCCAAAGGCCCGCAACTGGAAGCGCAACACAGCGCGGGC : 4341
Seqid3 : ..... : 4350
```

**Figure 1L**

4360 \*

Seqid1 : ATCAAATTAGGCTACCGCTGGTAA : 4365

Seqid3 : ..... : 4374

**Figure 2A**

Identity to SeqID No:2 is indicated by a dot, while a dash (-) indicates a nonexistent amino acid.

Seqid2 :	*                    20                    *	40                    *	
Seqid2 :	MKTTDKRTTETHRKAPKTGRIRFSPAYLAICLSFGILPQAWAGHTYFGIN :	50	
Seqid4 :	.....	50	
Seqid2 :	60                    *	80                    *	100
Seqid2 :	YQYYRDFAEKNGKFAVGAKDIEVYNKKGELVGKSMKAPMIDFSVVS RNG :	100	
Seqid4 :	.....	100	
Seqid2 :	*                    120                    *	140                    *	
Seqid2 :	VAALVGDQYIVSVAHNGGYNNVDFGAEGSNPDQHRFSYQIVKRNNYKAGT :	150	
Seqid4 :	.....R.....T.K.....	150	
Seqid2 :	160                    *	180                    *	200
Seqid2 :	NGHPYGGDYHMPRLHKFVTD AEPVEMTSYMDGRKYIDQNNYPDRVRIGAG :	200	
Seqid4 :	K.....	200	
Seqid2 :	*                    220                    *	240                    *	
Seqid2 :	RQYWRSEDEFPNNRESSYHIASAYSWLVGGNTFAQNGSGGGTVNLGSEKI :	250	
Seqid4 :	.....	250	
Seqid2 :	260                    *	280                    *	300
Seqid2 :	KHSPYGF LPTGGSFGDSGSPMFIYDAQKQKWLINGVLQTGNPYIGKSNGF :	300	
Seqid4 :	.....	300	
Seqid2 :	*                    320                    *	340                    *	
Seqid2 :	QLVRKDWFYDEIFAGDTHSVFYEPHQNGKYTFHDNNNGTGKINAKHEHNS :	350	
Seqid4 :	.....R.....S.N.D.....	350	

Figure 2B

```

          360          *          380          *          400
Seqid2 : LPNRLKTRTVQLFNVSLSETAREPVYHAAGGVNSYRPRLNNGENISFIDE : 400
Seqid4 : ..... : 400

          *          420          *          440          *
Seqid2 : GKGEILTSNINQGAGGLYFQGDFTVSPENNETWQGAGVHISEDSTVTWK : 450
Seqid4 : ..... : 450

          460          *          480          *          500
Seqid2 : VNGVANDRLSKIGKGT LHVQAKGENQGSISVGDGKVILDQQADENNKQA : 500
Seqid4 : .....T.....DKG... : 500

          *          520          *          540          *
Seqid2 : FSEIGLVSGRGT VQLNADNQFNPDKLYFGFRGGRDLNGHSLSFHRIQNT : 550
Seqid4 : ..... : 550

          560          *          580          *          600
Seqid2 : DEGAMIVNHNQDKESTVTITGNKDIATTGNNNSLDSKKELAYNGWFGEKD : 600
Seqid4 : ..... : 600

          *          620          *          640          *
Seqid2 : TTKTNGRLNLVYQPAAEDRTL LLSGGTNLNGNITQTNGKLFFSGRPTPHA : 650
Seqid4 : ..... : 650

          660          *          680          *          700
Seqid2 : YNHLGSGWSKMEGI PQGEIVWDNDWINRTFKAENFHIQGGQAVISRNVAK : 700
Seqid4 : ...NDH...QK...R.....Q.K...V..... : 700

          *          720          *          740          *
Seqid2 : VEGDWHLNHAQAVFGVAPHQSHTICTRSDWTGLTNCVEKTITDDKVIAS : 750
Seqid4 : .K..... : 750
```



Figure 2C

760                   \*                   780                   \*                   800  
Seqid2 : LTKTDISGNVSLADHAHLNLTGLATLNGNLSANGDTRYTVSHNATQNGDL : 800  
Seqid4 : .....D.....N. : 800

                 \*                   820                   \*                   840                   \*  
Seqid2 : SLVGNAQATFNQATLNGNTSASGNASFNLSMNAVQNGSLTSLGNAKANVS : 850  
Seqid4 : .....DH..... : 850

                 860                   \*                   880                   \*                   900  
Seqid2 : HSALNGNVSLADKAVFHFESSRFTGQISGSKDTALHLKDSEWTLPSGTEL : 900  
Seqid4 : .....G..... : 900

                 \*                   920                   \*                   940                   \*  
Seqid2 : GNLNLDNATITLNSAYRHDAAAGAQTSATDAPR---RRSRRSLLSVTPPA : 947  
Seqid4 : .....RRS.....T : 950

                 960                   \*                   980                   \*                   1000  
Seqid2 : SAESHFNLTFTVNGKLNQGTFRFMSELFGYRSDKLKLAESSECTYTLAVN : 997  
Seqid4 : .V..R..... : 1000

                 \*                   1020                   \*                   1040                   \*  
Seqid2 : NTGNEPVSLDQLTVVEGKDNKPLSENLFNFTLQNEHVDAGAWRYQLIRKDG : 1047  
Seqid4 : .....A..E.....F..... : 1050

                 1060                   \*                   1080                   \*                   1100  
Seqid2 : EFR LHNFVKEQELSDKLGKAEAKQAGKDNAQSLDALIAAGRDAVEKTES : 1097  
Seqid4 : .....E..... : 1100

                 \*                   1120                   \*                   1140                   \*  
Seqid2 : VAEPARQAGGENVGIMQAEEEKKRVQADKDTALAKQREGKTRPATTAFFPR : 1147  
Seqid4 : .....AE..... : 1150

## Figure 2D

Seqid2 :           1160           \*           1180           \*           1200  
Seqid4 : .....L..... : 1200

Seqid2 :           \*           1220           \*           1240           \*  
Seqid4 : ....E..... : 1250

Seqid2 :           1260           \*           1280           \*           1300  
Seqid4 : .....D..Y..A..... : 1300

Seqid2 :           \*           1320           \*           1340           \*  
Seqid4 : ...G..G..... : 1350

Seqid2 :           1360           \*           1380           \*           1400  
Seqid4 : ..... : 1400

Seqid2 :           \*           1420           \*           1440           \*  
Seqid4 : ..... : 1450

Seqid2 : IKLGYRW : 1454  
Seqid4 : ..... : 1457

Figure 3

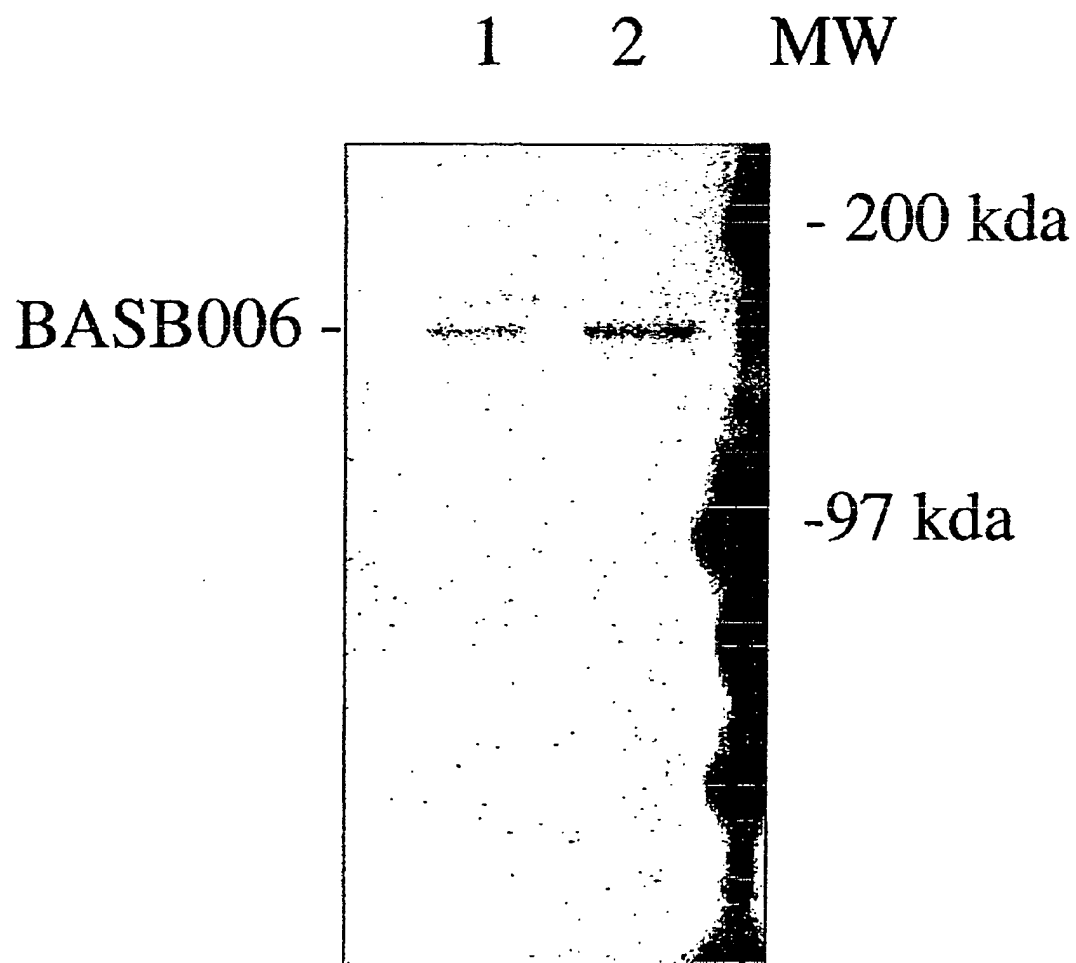
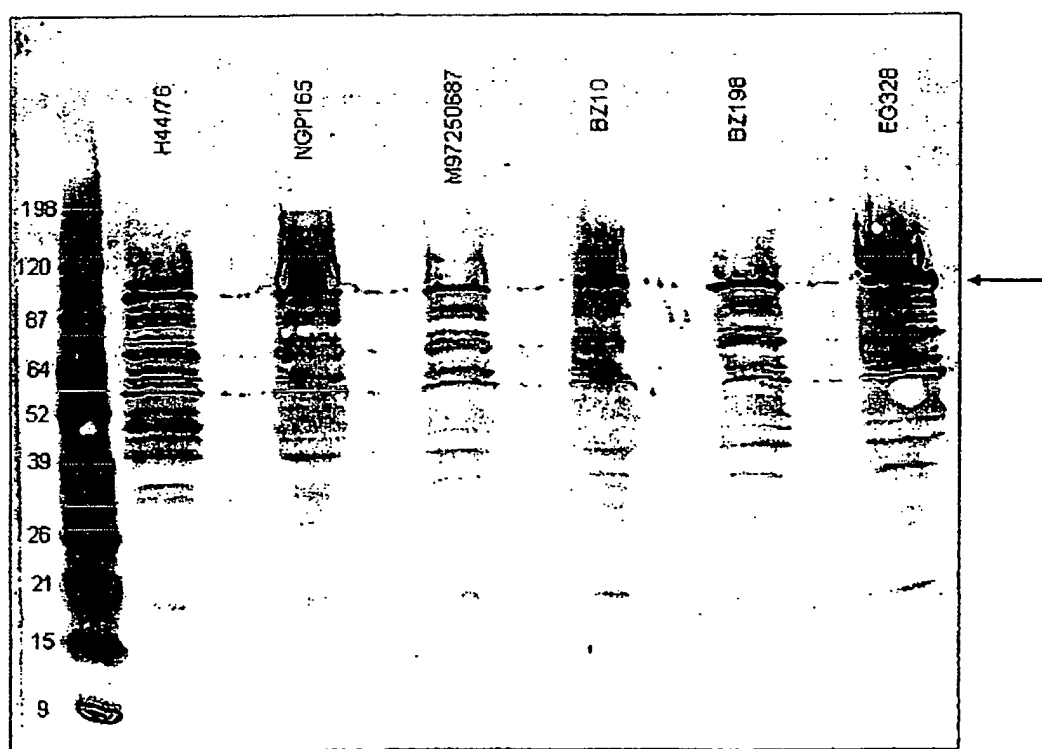
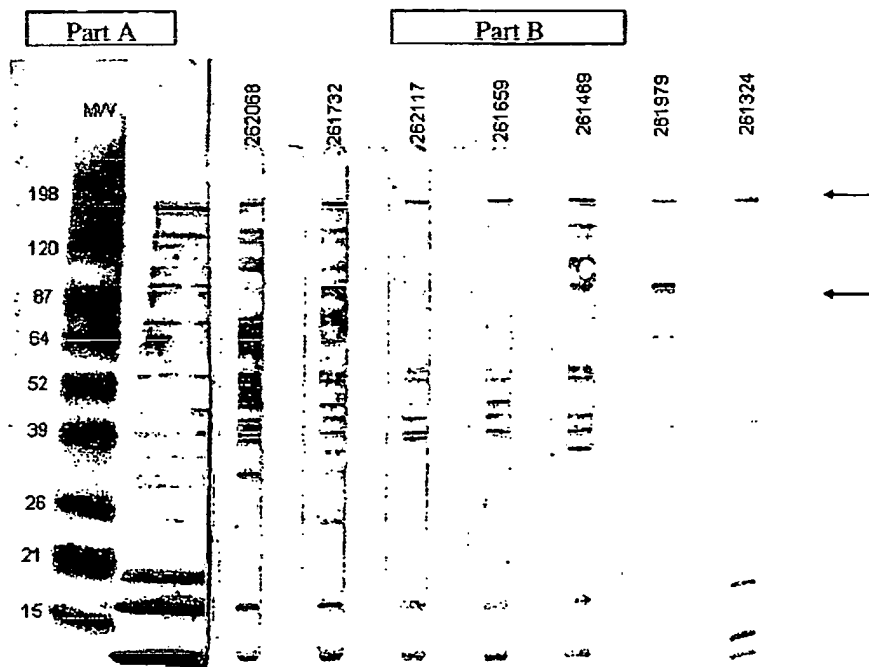


Figure 4



**Figure 5**

Anti-BASB006 antibodies in human convalescent sera (part B) and in immunized mice (part A).



# BASB006 POLYPEPTIDES FROM *NEISSERIA MENINGITIDIS* AND IMMUNOGENIC COMPOSITIONS THEREOF

## CROSS REFERENCE TO RELATED APPLICATIONS

This application is a divisional application of U.S. patent application Ser. No. 09/673,896 (which issued on Feb. 24, 2004 as U.S. Pat. No. 6,696,062), filed Dec. 18, 2000, which is the National Stage Application of International Application No. PCT/EP99/02766, filed Apr. 20, 1999 which was published under PCT article 21(2) in English, which claims the benefit of priority of Great Britain Patent Application Serial No. 9808866.9, filed Apr. 24, 1998. The disclosures of these applications are herein incorporated by reference in their entirety.

## FIELD OF THE INVENTION

This invention relates to polynucleotides, (herein referred to as "BASB006 polynucleotide(s)"), polypeptides encoded by them (referred to herein as "BASB006" or "BASB006 polypeptide(s)"), recombinant materials and methods for their production. In another aspect, the invention relates to methods for using such polypeptides and polynucleotides, including vaccines against bacterial infections. In a further aspect, the invention relates to diagnostic assays for detecting infection of certain pathogens.

## BACKGROUND OF THE INVENTION

*Neisseria meningitidis* (meningococcus) is a Gram negative bacterium frequently isolated from the human upper respiratory tract. It occasionally causes invasive bacterial diseases such as bacteremia and meningitis. The incidence of meningococcal disease shows geographical seasonal and annual differences (Schwartz, B., Moore, P. S., Broome, C. V.; Clin. Microbiol. Rev. 2 (Supplement), S18-S24, 1989). Most disease in temperate countries is due to strains of serogroup B and varies in incidence from 1-10/100,000/year total population sometimes reaching higher values (Kaczmarek, E. B. (1997), Commun. Dis. Rep. Rev. 7: R55-9, 1995; Scholten, R. J. P. M., Bijlmer, H. A., Poolman, J. T. et al. Clin. Infect. Dis. 16: 237-246, 1993; Cruz, C., Pavez, G., Aguilar, E., et al. Epidemiol. Infect. 105: 119-126, 1990).

Epidemics dominated by serogroup A meningococci, mostly in central Africa, are encountered, sometimes reaching levels up to 1000/100,000/year (Schwartz, B., Moore, P. S., Broome, C. V. Clin. Microbiol. Rev. 2 (Supplement), S18-S24, 1989). Nearly all cases as a whole of meningococcal disease are caused by serogroup A, B, C, W-135 and Y meningococci and a tetravalent A, C, W-135, Y polysaccharide vaccine is available (Armand, J., Arminjon, F., Mynard, M. C., Lafaix, C., J. Biol. Stand. 10: 335-339, 1982).

The polysaccharide vaccines are currently being improved by way of chemical conjugating them to carrier proteins (Lieberman, J. M., Chiu, S. S., Wong, V. K., et al. JAMA 275: 1499-1503, 1996).

A serogroup B vaccine is not available, since the B capsular polysaccharide was found to be nonimmunogenic, most likely because it shares structural similarity to host components (Wyle, F. A., Artenstein, M. S., Brandt, M. L. et al. J. Infect. Dis. 126: 514-522, 1972; Finne, J. M., Leinonen, M., Mäkelä, P. M. Lancet ii: 355-357, 1983).

For many years efforts have been initiated and carried out to develop meningococcal outer membrane based vaccines

(de Moraes, J. C., Perkins, B., Camargo, M. C. et al. Lancet 340: 1074-1078, 1992; Bjune, G., Hoiby, E. A. Gronnesby, J. K. et al. 338: 1093-1096, 1991). Such vaccines have demonstrated efficacies from 57%-85% in older children (>4 years) and adolescents.

Many bacterial outer membrane components are present in these vaccines, such as PorA, PorB, Rmp, Opc, Opa, FrpB and the contribution of these components to the observed protection still needs further definition. Other bacterial outer membrane components have been defined by using animal or human antibodies to be potentially relevant to the induction of protective immunity, such as TbpB and NspA (Martin, D., Cadieux, N., Hamel, J., Brodeux, B. R., J. Exp. Med. 185: 1173-1183, 1997; Lissolo, L., Maitre-Wilmotte, C., Dumas, P. et al., Inf. Immun. 63: 884-890, 1995). The mechanisms of protective immunity will involve antibody mediated bactericidal activity and opsonophagocytosis.

A bacteremia animal model has been used to combine all antibody mediated mechanisms (Saukkonen, K., Leinonen, M., Abdillahi, H. Poolman, J. T. Vaccine 7: 325-328, 1989). It is generally accepted that the late complement component mediated bactericidal mechanism is crucial for immunity against meningococcal disease (Ross, S. C., Rosenthal P. J., Berberic, H. M., Densen, P. J. Infect. Dis. 155: 1266-1275, 1987).

The frequency of *Neisseria meningitidis* infections has risen dramatically in the past few decades. This has been attributed to the emergence of multiply antibiotic resistant strains and an increasing population of people with weakened immune systems. It is no longer uncommon to isolate *Neisseria meningitidis* strains that are resistant to some or all of the standard antibiotics. This phenomenon has created an unmet medical need and demand for new anti-microbial agents, vaccines, drug screening methods, and diagnostic tests for this organism.

## SUMMARY OF THE INVENTION

The present invention relates to BASB006, in particular BASB006 polypeptides and BASB006 polynucleotides, recombinant materials and methods for their production. In another aspect, the invention relates to methods for using such polypeptides and polynucleotides, including prevention and treatment of microbial diseases, amongst others. In a further aspect, the invention relates to diagnostic assays for detecting diseases associated with microbial infections and conditions associated with such infections, such as assays for detecting expression or activity of BASB006 polynucleotides or polypeptides.

Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the following descriptions and from reading the other parts of the present disclosure.

## BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A-1L show consecutive segments of sequence alignment for two BASB006-encoding polynucleotides.

FIGS. 2A-2D show consecutive segments of sequence alignment for two BASB006 polypeptides.

FIG. 3 shows a Coomassie stain SDS-PAGE gel analysis of purified BASB006 protein.

FIG. 4 shows a Western-blot of partially purified recombinant BASB006 protein probed with mice sera containing anti-BASB006 antibodies.

FIG. 5 shows a Western-blot of partially purified recombinant BASB006 protein probed with human convalescent sera and mice sera.

### DESCRIPTION OF THE INVENTION

The invention relates to BASB006 polypeptides and polynucleotides as described in greater detail below. In particular, the invention relates to polypeptides and polynucleotides of BASB006 of *Neisseria meningitidis*, which is related by amino acid sequence homology to *H. influenzae* Hap polypeptide. The invention relates especially to BASB006 having the nucleotide and amino acid sequences set out in SEQ ID NO:1,3 and SEQ ID NO:2,4 respectively. It is understood that sequences recited in the Sequence Listing below as "DNA" represent an exemplification of one embodiment of the invention, since those of ordinary skill will recognize that such sequences can be usefully employed in polynucleotides in general, including ribopolynucleotides.

#### Polypeptides

In one aspect of the invention there are provided polypeptides of *Neisseria meningitidis* referred to herein as "BASB006" and "BASB006 polypeptides" as well as biologically, diagnostically, prophylactically, clinically or therapeutically useful variants thereof, and compositions comprising the same.

The present invention further provides for:

- (a) an isolated polypeptide which comprises an amino acid sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% or exact identity, to that of SEQ ID NO:2, 4;
- (b) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to SEQ ID NO:1, 3 over the entire length of SEQ ID NO:1, 3 respectively; or
- (c) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence encoding a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity, to the amino acid sequence of SEQ ID NO:2, 4;

The BASB006 polypeptides provided in SEQ ID NO:2,4 are the BASB006 polypeptides from *Neisseria meningitidis* strains American Type Culture Collection 13090 (herein "ATCC13090") and H44/76.

The invention also provides an immunogenic fragment of a BASB006 polypeptide, that is, a contiguous portion of the BASB006 polypeptide which has the same or substantially the same immunogenic activity as the polypeptide comprising the amino acid sequence of SEQ ID NO:2,4. That is to say, the fragment (if necessary when coupled to a carrier) is capable of raising an immune response which recognises the BASB006 polypeptide. Such an immunogenic fragment may include, for example, the BASB006 polypeptide lacking an N-terminal leader sequence, and/or a transmembrane domain and/or a C-terminal anchor domain. In a preferred aspect the immunogenic fragment of BASB006 according to the invention comprises substantially all of the extracellular domain of a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% identity, to that of SEQ ID NO:2,4 over the entire length of SEQ ID NO:2.

A fragment is a polypeptide having an amino acid sequence that is entirely the same as part but not all of any amino acid sequence of any polypeptide of the invention. As with BASB006 polypeptides, fragments may be "free-standing," or comprised within a larger polypeptide of which they form a part or region, most preferably as a single continuous region in a single larger polypeptide.

Preferred fragments include, for example, truncation polypeptides having a portion of an amino acid sequence of SEQ ID NO:2,4 or of variants thereof, such as a continuous series of residues that includes an amino- and/or carboxyl-terminal amino acid sequence. Degradation forms of the polypeptides of the invention produced by or in a host cell, are also preferred. Further preferred are fragments characterized by structural or functional attributes such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions.

Further preferred fragments include an isolated polypeptide comprising an amino acid sequence having at least 15, 20, 30, 40, 50 or 100 contiguous amino acids from the amino acid sequence of SEQ ID NO:2,4, or an isolated polypeptide comprising an amino acid sequence having at least 15, 20, 30, 40, 50 or 100 contiguous amino acids truncated or deleted from the amino acid sequence of SEQ ID NO:2,4.

Fragments of the polypeptides of the invention may be employed for producing the corresponding full-length polypeptide by peptide synthesis; therefore, these fragments may be employed as intermediates for producing the full-length polypeptides of the invention.

Particularly preferred are variants in which several, 5-10, 1-5, 1-3, 1-2 or 1 amino acids are substituted, deleted, or added in any combination.

The polypeptides, or immunogenic fragments, of the invention may be in the form of the "mature" protein or may be a part of a larger protein such as a precursor or a fusion protein. It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification such as multiple histidine residues, or an additional sequence for stability during recombinant production. Furthermore, addition of exogenous polypeptide or lipid tail or polynucleotide sequences to increase the immunogenic potential of the final molecule is also considered.

In one aspect, the invention relates to genetically engineered soluble fusion proteins comprising a polypeptide of the present invention, or a fragment thereof, and various portions of the constant regions of heavy or light chains of immunoglobulins of various subclasses (IgG, IgM, IgA, IgE). Preferred as an immunoglobulin is the constant part of the heavy chain of human IgG, particularly IgG1, where fusion takes place at the hinge region. In a particular embodiment, the Fc part can be removed simply by incorporation of a cleavage sequence which can be cleaved with blood clotting factor Xa.

Furthermore, this invention relates to processes for the preparation of these fusion proteins by genetic engineering, and to the use thereof for drug screening, diagnosis and therapy. A further aspect of the invention also relates to polynucleotides encoding such fusion proteins. Examples of fusion protein technology can be found in International Patent Application Nos. WO94/29458 and WO94/22914.

The proteins may be chemically conjugated, or expressed as recombinant fusion proteins allowing increased levels to

be produced in an expression system as compared to non-fused protein. The fusion partner may assist in providing T helper epitopes (immunological fusion partner), preferably T helper epitopes recognised by humans, or assist in expressing the protein (expression enhancer) at higher yields than the native recombinant protein. Preferably the fusion partner will be both an immunological fusion partner and expression enhancing partner.

Fusion partners include protein D from *Haemophilus influenzae* and the non-structural protein from influenzae virus, NS 1 (hemagglutinin). Another fusion partner is the protein known as LytA. Preferably the C terminal portion of the molecule is used. LytA is derived from *Streptococcus pneumoniae* which synthesize an N-acetyl-L-alanine amidase, amidase LytA, (coded by the lytA gene {Gene, 43 (1986) page 265-272}) an autolysin that specifically degrades certain bonds in the peptidoglycan backbone. The C-terminal domain of the LytA protein is responsible for the affinity to the choline or to some choline analogues such as DEAE. This property has been exploited for the development of *E. coli* C-LytA expressing plasmids useful for expression of fusion proteins. Purification of hybrid proteins containing the C-LytA fragment at its amino terminus has been described {Biotechnology: 10, (1992) page 795-798}. It is possible to use the repeat portion of the LytA molecule found in the C terminal end starting at residue 178, for example residues 188-305.

The present invention also includes variants of the aforementioned polypeptides, that is polypeptides that vary from the referents by conservative amino acid substitutions, whereby a residue is substituted by another with like characteristics. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; and among the basic residues Lys and Arg; or aromatic residues Phe and Tyr.

Polypeptides of the present invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

It is most preferred that a polypeptide of the invention is derived from *Neisseria meningitidis*, however, it may preferably be obtained from other organisms of the same taxonomic genus. A polypeptide of the invention may also be obtained, for example, from organisms of the same taxonomic family or order.

#### Polynucleotides

It is an object of the invention to provide polynucleotides that encode BASB006 polypeptides, particularly polynucleotides that encode the polypeptide herein designated BASB006.

In a particularly preferred embodiment of the invention the polynucleotide comprises a region encoding BASB006 polypeptides comprising a sequence set out in SEQ ID NO:1,3 which includes a full length gene, or a variant thereof.

The BASB006 polynucleotides provided in SEQ ID NO:1,3 are the BASB006 polynucleotides from *Neisseria meningitidis* strains ATCC13090 and H44/76.

As a further aspect of the invention there are provided isolated nucleic acid molecules encoding and/or expressing BASB006 polypeptides and polynucleotides, particularly *Neisseria meningitidis* BASB006 polypeptides and polynucleotides, including, for example, unprocessed RNAs, ribozyme RNAs, mRNAs, cDNAs, genomic DNAs, B- and

Z-DNAs. Further embodiments of the invention include biologically, diagnostically, prophylactically, clinically or therapeutically useful polynucleotides and polypeptides, and variants thereof, and compositions comprising the same.

Another aspect of the invention relates to isolated polynucleotides, including at least one full length gene, that encodes a BASB006 polypeptide having a deduced amino acid sequence of SEQ ID NO:2,4 and polynucleotides closely related thereto and variants thereof.

In another particularly preferred embodiment of the invention there is a BASB006 polypeptide from *Neisseria meningitidis* comprising or consisting of an amino acid sequence of SEQ ID NO:2,4 or a variant thereof.

Using the information provided herein, such as a polynucleotide sequence set out in SEQ ID NO:1, 3 a polynucleotide of the invention encoding BASB006 polypeptide may be obtained using standard cloning and screening methods, such as those for cloning and sequencing chromosomal DNA fragments from bacteria using *Neisseria meningitidis* cells as starting material, followed by obtaining a full length clone. For example, to obtain a polynucleotide sequence of the invention, such as a polynucleotide sequence given in SEQ ID NO:1,3, typically a library of clones of chromosomal DNA of *Neisseria meningitidis* in *E. coli* or some other suitable host is probed with a radiolabeled oligonucleotide, preferably a 17-mer or longer, derived from a partial sequence. Clones carrying DNA identical to that of the probe can then be distinguished using stringent hybridization conditions. By sequencing the individual clones thus identified by hybridization with sequencing primers designed from the original polypeptide or polynucleotide sequence it is then possible to extend the polynucleotide sequence in both directions to determine a full length gene sequence. Conveniently, such sequencing is performed, for example, using denatured double stranded DNA prepared from a plasmid clone. Suitable techniques are described by Maniatis, T., Fritsch, E. F. and Sambrook et al., *MOLECULAR CLONING, A LABORATORY MANUAL*, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989). (see in particular Screening By Hybridization 1.90 and Sequencing Denatured Double-Stranded DNA Templates 13.70). Direct genomic DNA sequencing may also be performed to obtain a full length gene sequence. Illustrative of the invention, each polynucleotide set out in SEQ ID NO:1,3 was discovered in a DNA library derived from *Neisseria meningitidis*.

Moreover, each DNA sequence set out in SEQ ID NO:1,3 contains an open reading frame encoding a protein having about the number of amino acid residues set forth in SEQ ID NO:2, 4 with a deduced molecular weight that can be calculated using amino acid residue molecular weight values well known to those skilled in the art.

The polynucleotide of SEQ ID NO:1, between the start codon at nucleotide number 1 and the stop codon which begins at nucleotide number 4363 of SEQ ID NO:1, encodes the polypeptide of SEQ ID NO:2.

The polynucleotide of SEQ ID NO:3, between the start codon at nucleotide number 1 and the stop codon which begins at nucleotide number 4372 of SEQ ID NO:3, encodes the polypeptide of SEQ ID NO:4.

In a further aspect, the present invention provides for an isolated polynucleotide comprising or consisting of:

- (a) a polynucleotide sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to SEQ ID NO:1,3 over the entire length of SEQ ID NO:1,3 respectively; or



(b) a polynucleotide sequence encoding a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or 100% exact, to the amino acid sequence of SEQ ID NO:2, 4 over the entire length of SEQ ID NO:2, 4 respectively.

A polynucleotide encoding a polypeptide of the present invention, including homologs and orthologs from species other than *Neisseria meningitidis*, may be obtained by a process which comprises the steps of screening an appropriate library under stringent hybridization conditions (for example, using a temperature in the range of 45-65° C. and an SDS concentration from 0.1-1%) with a labeled or detectable probe consisting of or comprising the sequence of SEQ ID NO:1, 3 or a fragment thereof; and isolating a full-length gene and/or genomic clones containing said polynucleotide sequence.

The invention provides a polynucleotide sequence identical over its entire length to a coding sequence (open reading frame) in SEQ ID NO:1, 3. Also provided by the invention is a coding sequence for a mature polypeptide or a fragment thereof, by itself as well as a coding sequence for a mature polypeptide or a fragment in reading frame with another coding sequence, such as a sequence encoding a leader or secretory sequence, a pre-, or pro- or prepro-protein sequence. The polynucleotide of the invention may also contain at least one non-coding sequence, including for example, but not limited to at least one non-coding 5' and 3' sequence, such as the transcribed but non-translated sequences, termination signals (such as rho-dependent and rho-independent termination signals), ribosome binding sites, Kozak sequences, sequences that stabilize mRNA, introns, and polyadenylation signals. The polynucleotide sequence may also comprise additional coding sequence encoding additional amino acids. For example, a marker sequence that facilitates purification of the fused polypeptide can be encoded. In certain embodiments of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz et al., *Proc. Natl. Acad. Sci., USA* 86: 821-824 (1989), or an HA peptide tag (Wilson et al., *Cell* 37: 767 (1984), both of which may be useful in purifying polypeptide sequence fused to them. Polynucleotides of the invention also include, but are not limited to, polynucleotides comprising a structural gene and its naturally associated sequences that control gene expression.

The nucleotide sequence encoding BASB006 polypeptide of SEQ ID NO:2, 4 may be identical to the polypeptide encoding sequence contained in nucleotides 1 to 4362 of SEQ ID NO:1, or the polypeptide encoding sequence contained in nucleotides 1 to 4371 of SEQ ID NO:3, respectively. Alternatively it may be a sequence, which as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:2, 4.

The term "polynucleotide encoding a polypeptide" as used herein encompasses polynucleotides that include a sequence encoding a polypeptide of the invention, particularly a bacterial polypeptide and more particularly a polypeptide of the *Neisseria meningitidis* BASB006 having an amino acid sequence set out in SEQ ID NO:2, 4. The term also encompasses polynucleotides that include a single continuous region or discontinuous regions encoding the polypeptide (for example, polynucleotides interrupted by integrated phage, an integrated insertion sequence, an integrated vector sequence, an integrated transposon sequence, or due to RNA editing or genomic DNA reorganization) together with additional regions, that also may contain coding and/or non-coding sequences.

The invention further relates to variants of the polynucleotides described herein that encode variants of a polypeptide having a deduced amino acid sequence of SEQ ID NO:2, 4. Fragments of polynucleotides of the invention may be used, for example, to synthesize full-length polynucleotides of the invention.

Further particularly preferred embodiments are polynucleotides encoding BASB006 variants, that have the amino acid sequence of BASB006 polypeptide of SEQ ID NO:2, 4 in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, modified, deleted and/or added, in any combination. Especially preferred among these are silent substitutions, additions and deletions, that do not alter the properties and activities of BASB006 polypeptide.

Further preferred embodiments of the invention are polynucleotides that are at least 85% identical over their entire length to a polynucleotide encoding BASB006 polypeptide having an amino acid sequence set out in SEQ ID NO:2, 4, and polynucleotides that are complementary to such polynucleotides. In this regard, polynucleotides at least 90% identical over their entire length to the same are particularly preferred, and among these particularly preferred polynucleotides, those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred among those with at least 95%, and among these those with at least 98% and at least 99% are particularly highly preferred, with at least 99% being the more preferred.

Preferred embodiments are polynucleotides encoding polypeptides that retain substantially the same biological function or activity as the mature polypeptide encoded by a DNA of SEQ ID NO:1, 3.

In accordance with certain preferred embodiments of this invention there are provided polynucleotides that hybridize, particularly under stringent conditions, to BASB006 polynucleotide sequences, such as those polynucleotides in SEQ ID NO:1, 3.

The invention further relates to polynucleotides that hybridize to the polynucleotide sequences provided herein. In this regard, the invention especially relates to polynucleotides that hybridize under stringent conditions to the polynucleotides described herein. As herein used, the terms "stringent conditions" and "stringent hybridization conditions" mean hybridization occurring only if there is at least 95% and preferably at least 97% identity between the sequences. A specific example of stringent hybridization conditions is overnight incubation at 42° C. in a solution comprising: 50% formamide, 5×SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5×Denhardt's solution, 10% dextran sulfate, and 20 micrograms/ml of denatured, sheared salmon sperm DNA, followed by washing the hybridization support in 0.1×SSC at about 65° C. Hybridization and wash conditions are well known and exemplified in Sambrook, et al., *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor, N.Y., (1989), particularly Chapter 11 therein. Solution hybridization may also be used with the polynucleotide sequences provided by the invention.

The invention also provides a polynucleotide consisting of or comprising a polynucleotide sequence obtained by screening an appropriate library containing the complete gene for a polynucleotide sequence set forth in SEQ ID NO:1, 3 under stringent hybridization conditions with a probe having the sequence of said polynucleotide sequence set forth in SEQ ID NO:1, 3 or a fragment thereof, and isolating said polynucleotide sequence. Fragments useful for obtaining such a polynucleotide include, for example, probes and primers fully described elsewhere herein.

As discussed elsewhere herein regarding polynucleotide assays of the invention, for instance, the polynucleotides of the invention, may be used as a hybridization probe for RNA, cDNA and genomic DNA to isolate full-length cDNAs and genomic clones encoding BASB006 and to isolate cDNA and genomic clones of other genes that have a high identity, particularly high sequence identity, to the BASB006 gene. Such probes generally will comprise at least 15 nucleotide residues or base pairs. Preferably, such probes will have at least 30 nucleotide residues or base pairs and may have at least 50 nucleotide residues or base pairs. Particularly preferred probes will have at least 20 nucleotide residues or base pairs and will have less than 30 nucleotide residues or base pairs.

A coding region of a BASB006 gene may be isolated by screening using a DNA sequence provided in SEQ ID NO:1, 3 to synthesize an oligonucleotide probe. A labeled oligonucleotide having a sequence complementary to that of a gene of the invention is then used to screen a library of cDNA, genomic DNA or mRNA to determine which members of the library the probe hybridizes to.

There are several methods available and well known to those skilled in the art to obtain full-length DNAs, or extend short DNAs, for example those based on the method of Rapid Amplification of cDNA ends (RACE) (see, for example, Frohman, et al., *PNAS USA* 85: 8998-9002, 1988). Recent modifications of the technique, exemplified by the Marathon™ technology (Clontech Laboratories Inc.) for example, have significantly simplified the search for longer cDNAs. In the Marathon™ technology, cDNAs have been prepared from mRNA extracted from a chosen tissue and an 'adaptor' sequence ligated onto each end. Nucleic acid amplification (PCR) is then carried out to amplify the "missing" 5' end of the DNA using a combination of gene specific and adaptor specific oligonucleotide primers. The PCR reaction is then repeated using "nested" primers, that is, primers designed to anneal within the amplified product (typically an adaptor specific primer that anneals further 3' in the adaptor sequence and a gene specific primer that anneals further 5' in the selected gene sequence). The products of this reaction can then be analyzed by DNA sequencing and a full-length DNA constructed either by joining the product directly to the existing DNA to give a complete sequence, or carrying out a separate full-length PCR using the new sequence information for the design of the 5' primer.

The polynucleotides and polypeptides of the invention may be employed, for example, as research reagents and materials for discovery of treatments of and diagnostics for diseases, particularly human diseases, as further discussed herein relating to polynucleotide assays.

The polynucleotides of the invention that are oligonucleotides derived from a sequence of SEQ ID NOS:1-4 may be used in the processes herein as described, but preferably for PCR, to determine whether or not the polynucleotides identified herein in whole or in part are transcribed in bacteria in infected tissue. It is recognized that such sequences will also have utility in diagnosis of the stage of infection and type of infection the pathogen has attained.

The invention also provides polynucleotides that encode a polypeptide that is the mature protein plus additional amino or carboxyl-terminal amino acids, or amino acids interior to the mature polypeptide (when the mature form has more than one polypeptide chain, for instance). Such sequences may play a role in processing of a protein from precursor to a mature form, may allow protein transport, may lengthen or shorten protein half-life or may facilitate manipulation of a protein for assay or production, among other things. As gen-

erally is the case in vivo, the additional amino acids may be processed away from the mature protein by cellular enzymes.

For each and every polynucleotide of the invention there is provided a polynucleotide complementary to it. It is preferred that these complementary polynucleotides are fully complementary to each polynucleotide with which they are complementary.

A precursor protein, having a mature form of the polypeptide fused to one or more prosequences may be an inactive form of the polypeptide. When prosequences are removed such inactive precursors generally are activated. Some or all of the prosequences may be removed before activation. Generally, such precursors are called proproteins.

In addition to the standard A, G, C, T/U representations for nucleotides, the term "N" may also be used in describing certain polynucleotides of the invention. "N" means that any of the four DNA or RNA nucleotides may appear at such a designated position in the DNA or RNA sequence, except it is preferred that N is not a nucleic acid that when taken in combination with adjacent nucleotide positions, when read in the correct reading frame, would have the effect of generating a premature termination codon in such reading frame.

In sum, a polynucleotide of the invention may encode a mature protein, a mature protein plus a leader sequence (which may be referred to as a preprotein), a precursor of a mature protein having one or more prosequences that are not the leader sequences of a preprotein, or a preprotein, which is a precursor to a proprotein, having a leader sequence and one or more prosequences, which generally are removed during processing steps that produce active and mature forms of the polypeptide.

In accordance with an aspect of the invention, there is provided the use of a polynucleotide of the invention for therapeutic or prophylactic purposes, in particular genetic immunization.

The use of a polynucleotide of the invention in genetic immunization will preferably employ a suitable delivery method such as direct injection of plasmid DNA into muscles (Wolff et al., *Hum Mol Genet* (1992) 1: 363, Manthorpe et al., *Hum. Gene Ther.* (1983) 4: 419), delivery of DNA complexed with specific protein carriers (Wu et al., *J Biol Chem.* (1989) 264: 16985), coprecipitation of DNA with calcium phosphate (Benvenisty & Reshef, *PNAS USA*, (1986) 83: 9551), encapsulation of DNA in various forms of liposomes (Kaneda et al., *Science* (1989) 243: 375), particle bombardment (Tang et al., *Nature* (1992) 356:152, Eisenbraun et al., *DNA Cell Biol* (1993) 12: 791) and in vivo infection using cloned retroviral vectors (Seeger et al., *PNAS USA* (1984) 81: 5849).

#### 50 Vectors, Host Cells, Expression Systems

The invention also relates to vectors that comprise a polynucleotide or polynucleotides of the invention, host cells that are genetically engineered with vectors of the invention and the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the invention.

Recombinant polypeptides of the present invention may be prepared by processes well known in those skilled in the art from genetically engineered host cells comprising expression systems. Accordingly, in a further aspect, the present invention relates to expression systems that comprise a polynucleotide or polynucleotides of the present invention, to host cells which are genetically engineered with such expression systems, and to the production of polypeptides of the invention by recombinant techniques.

For recombinant production of the polypeptides of the invention, host cells can be genetically engineered to incorporate expression systems or portions thereof or polynucleotides of the invention. Introduction of a polynucleotide into the host cell can be effected by methods described in many standard laboratory manuals, such as Davis, et al., *BASIC METHODS IN MOLECULAR BIOLOGY*, (1986) and Sambrook, et al., *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989), such as, calcium phosphate transfection, DEAE-dextran mediated transfection, transfection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction and infection.

Representative examples of appropriate hosts include bacterial cells, such as cells of streptococci, staphylococci, enterococci, *E. coli*, streptomyces, cyanobacteria, *Bacillus subtilis*, *Moraxella catarrhalis*, *Haemophilus influenzae* and *Neisseria meningitidis*; fungal cells, such as cells of a yeast, *Kluyveromyces*, *Saccharomyces*, a basidiomycete, *Candida albicans* and *Aspergillus*; insect cells such as cells of *Drosophila* S2 and *Spodoptera* Sf9; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, 293, CV-1 and Bowes melanoma cells; and plant cells, such as cells of a gymnosperm or angiosperm.

A great variety of expression systems can be used to produce the polypeptides of the invention. Such vectors include, among others, chromosomal-, episomal- and virus-derived vectors, for example, vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses, picornaviruses, retroviruses, and alphaviruses and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. The expression system constructs may contain control regions that regulate as well as engender expression. Generally, any system or vector suitable to maintain, propagate or express polynucleotides and/or to express a polypeptide in a host may be used for expression in this regard. The appropriate DNA sequence may be inserted into the expression system by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook et al., *MOLECULAR CLONING, A LABORATORY MANUAL*, (supra).

In recombinant expression systems in eukaryotes, for secretion of a translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the expressed polypeptide. These signals may be endogenous to the polypeptide or they may be heterologous signals.

Polypeptides of the present invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, ion metal affinity chromatography (IMAC) is employed for purification. Well known techniques for refolding proteins may be employed to regenerate active conformation when the polypeptide is denatured during intracellular synthesis, isolation and/or purification.

The expression system may also be a recombinant live microorganism, such as a virus or bacterium. The gene of

interest can be inserted into the genome of a live recombinant virus or bacterium. Inoculation and in vivo infection with this live vector will lead to in vivo expression of the antigen and induction of immune responses. Viruses and bacteria used for this purpose are for instance: poxviruses (e.g; vaccinia, fowlpox, canarypox), alphaviruses (Sindbis virus, Semliki Forest Virus, Venezuelan Equine Encephalitis Virus), adenoviruses, adeno-associated virus, picornaviruses (poliovirus, rhinovirus), herpesviruses (varicella zoster virus, etc), *Listeria*, *Salmonella*, *Shigella*, *Neisseria*, BCG. These viruses and bacteria can be virulent, or attenuated in various ways in order to obtain live vaccines. Such live vaccines also form part of the invention.

#### Diagnostic, Prognostic, Serotyping and Mutation Assays

This invention is also related to the use of BASB006 polynucleotides and polypeptides of the invention for use as diagnostic reagents. Detection of BASB006 polynucleotides and/or polypeptides in a eukaryote, particularly a mammal, and especially a human, will provide a diagnostic method for diagnosis of disease, staging of disease or response of an infectious organism to drugs. Eukaryotes, particularly mammals, and especially humans, particularly those infected or suspected to be infected with an organism comprising the BASB006 gene or protein, may be detected at the nucleic acid or amino acid level by a variety of well known techniques as well as by methods provided herein.

Polypeptides and polynucleotides for prognosis, diagnosis or other analysis may be obtained from a putatively infected and/or infected individual's bodily materials. Polynucleotides from any of these sources, particularly DNA or RNA, may be used directly for detection or may be amplified enzymatically by using PCR or any other amplification technique prior to analysis. RNA, particularly mRNA, cDNA and genomic DNA may also be used in the same ways. Using amplification, characterization of the species and strain of infectious or resident organism present in an individual, may be made by an analysis of the genotype of a selected polynucleotide of the organism. Deletions and insertions can be detected by a change in size of the amplified product in comparison to a genotype of a reference sequence selected from a related organism, preferably a different species of the same genus or a different strain of the same species. Point mutations can be identified by hybridizing amplified DNA to labeled BASB006 polynucleotide sequences. Perfectly or significantly matched sequences can be distinguished from imperfectly or more significantly mismatched duplexes by DNase or RNase digestion, for DNA or RNA respectively, or by detecting differences in melting temperatures or renaturation kinetics. Polynucleotide sequence differences may also be detected by alterations in the electrophoretic mobility of polynucleotide fragments in gels as compared to a reference sequence. This may be carried out with or without denaturing agents. Polynucleotide differences may also be detected by direct DNA or RNA sequencing. See, for example, Myers et al., *Science*, 230: 1242 (1985). Sequence changes at specific locations also may be revealed by nuclease protection assays, such as RNase, V1 and S1 protection assay or a chemical cleavage method. See, for example, Cotton et al., *Proc. Natl. Acad. Sci., USA*, 85: 4397-4401 (1988).

In another embodiment, an array of oligonucleotides probes comprising BASB006 nucleotide sequence or fragments thereof can be constructed to conduct efficient screening of, for example, genetic mutations, serotype, taxonomic classification or identification. Array technology methods are well known and have general applicability and can be used to address a variety of questions in molecular genetics including

gene expression, genetic linkage, and genetic variability (see, for example, Chee et al., *Science*, 274: 610 (1996)).

Thus in another aspect, the present invention relates to a diagnostic kit which comprises:

- (a) a polynucleotide of the present invention, preferably the nucleotide sequence of SEQ ID NO:1, 3, or a fragment thereof;
- (b) a nucleotide sequence complementary to that of (a);
- (c) a polypeptide of the present invention, preferably the polypeptide of SEQ ID NO:2, 4 or a fragment thereof, or
- (d) an antibody to a polypeptide of the present invention, preferably to the polypeptide of SEQ ID NO:2, 4.

It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component. Such a kit will be of use in diagnosing a disease or susceptibility to a disease, among others.

This invention also relates to the use of polynucleotides of the present invention as diagnostic reagents. Detection of a mutated form of a polynucleotide of the invention, preferable, SEQ ID NO:1, 3, which is associated with a disease or pathogenicity will provide a diagnostic tool that can add to, or define, a diagnosis of a disease, a prognosis of a course of disease, a determination of a stage of disease, or a susceptibility to a disease, which results from under-expression, over-expression or altered expression of the polynucleotide. Organisms, particularly infectious organisms, carrying mutations in such polynucleotide may be detected at the polynucleotide level by a variety of techniques, such as those described elsewhere herein.

Cells from an organism carrying mutations or polymorphisms (allelic variations) in a polynucleotide and/or polypeptide of the invention may also be detected at the polynucleotide or polypeptide level by a variety of techniques, to allow for serotyping, for example. For example, RT-PCR can be used to detect mutations in the RNA. It is particularly preferred to use RT-PCR in conjunction with automated detection systems, such as, for example, GeneScan. RNA, cDNA or genomic DNA may also be used for the same purpose, PCR. As an example, PCR primers complementary to a polynucleotide encoding BASB006 polypeptide can be used to identify and analyze mutations.

The invention further provides primers with 1, 2, 3 or 4 nucleotides removed from the 5' and/or the 3' end. These primers may be used for, among other things, amplifying BASB006 DNA and/or RNA isolated from a sample derived from an individual, such as a bodily material. The primers may be used to amplify a polynucleotide isolated from an infected individual, such that the polynucleotide may then be subject to various techniques for elucidation of the polynucleotide sequence. In this way, mutations in the polynucleotide sequence may be detected and used to diagnose and/or prognose the infection or its stage or course, or to serotype and/or classify the infectious agent.

The invention further provides a process for diagnosing disease, preferably bacterial infections, more preferably infections caused by *Neisseria meningitidis*, comprising determining from a sample derived from an individual, such as a bodily material, an increased level of expression of polynucleotide having a sequence of SEQ ID NO:1, 3. Increased or decreased expression of a BASB006 polynucleotide can be measured using any one of the methods well known in the art for the quantitation of polynucleotides, such as, for example, amplification, PCR, RT-PCR, RNase protection, Northern blotting, spectrometry and other hybridization methods.

In addition, a diagnostic assay in accordance with the invention for detecting over-expression of BASB006 polypeptide compared to normal control tissue samples may

be used to detect the presence of an infection, for example. Assay techniques that can be used to determine levels of a BASB006 polypeptide, in a sample derived from a host, such as a bodily material, are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis, antibody sandwich assays, antibody detection and ELISA assays.

The polynucleotides of the invention may be used as components of polynucleotide arrays, preferably high density arrays or grids. These high density arrays are particularly useful for diagnostic and prognostic purposes. For example, a set of spots each comprising a different gene, and further comprising a polynucleotide or polynucleotides of the invention, may be used for probing, such as using hybridization or nucleic acid amplification, using a probe obtained or derived from a bodily sample, to determine the presence of a particular polynucleotide sequence or related sequence in an individual. Such a presence may indicate the presence of a pathogen, particularly *Neisseria meningitidis*, and may be useful in diagnosing and/or prognosing disease or a course of disease. A grid comprising a number of variants of the polynucleotide sequence of SEQ ID NO:1, 3 are preferred. Also preferred is a grid comprising a number of variants of a polynucleotide sequence encoding the polypeptide sequence of SEQ ID NO:2, 4.

#### Antibodies

The polypeptides and polynucleotides of the invention or variants thereof, or cells expressing the same can be used as immunogens to produce antibodies immunospecific for such polypeptides or polynucleotides respectively.

In certain preferred embodiments of the invention there are provided antibodies against BASB006 polypeptides or polynucleotides.

Antibodies generated against the polypeptides or polynucleotides of the invention can be obtained by administering the polypeptides and/or polynucleotides of the invention, or epitope-bearing fragments of either or both, analogues of either or both, or cells expressing either or both, to an animal, preferably a nonhuman, using routine protocols. For preparation of monoclonal antibodies, any technique known in the art that provides antibodies produced by continuous cell line cultures can be used. Examples include various techniques, such as those in Kohler, G. and Milstein, C., *Nature* 256: 495-497 (1975); Kozbor et al., *Immunology Today* 4: 72 (1983); Cole et al., pg. 77-96 in *MONOCLONAL ANTIBODIES AND CANCER THERAPY*, Alan R. Liss, Inc. (1985).

Techniques for the production of single chain antibodies (U.S. Pat. No. 4,946,778) can be adapted to produce single chain antibodies to polypeptides or polynucleotides of this invention. Also, transgenic mice, or other organisms or animals, such as other mammals, may be used to express humanized antibodies immunospecific to the polypeptides or polynucleotides of the invention.

Alternatively, phage display technology may be utilized to select antibody genes with binding activities towards a polypeptide of the invention either from repertoires of PCR amplified v-genes of lymphocytes from humans screened for possessing anti-BASB006 or from naive libraries (McCafferty, et al., (1990), *Nature* 348, 552-554; Marks, et al., (1992) *Biotechnology* 10, 779-783). The affinity of these antibodies can also be improved by, for example, chain shuffling (Clackson et al., (1991) *Nature* 352: 628).

The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptides or polynucleotides of the invention to purify the polypeptides or polynucleotides by, for example, affinity chromatography.

Thus, among others, antibodies against BASB006-polypeptide or BASB006-polynucleotide may be employed to treat infections, particularly bacterial infections.

Polypeptide variants include antigenically, epitopically or immunologically equivalent variants form a particular aspect of this invention.

Preferably, the antibody or variant thereof is modified to make it less immunogenic in the individual. For example, if the individual is human the antibody may most preferably be "humanized," where the complementarily determining region or regions of the hybridoma-derived antibody has been transplanted into a human monoclonal antibody, for example as described in Jones et al. (1986), *Nature* 321, 522-525 or Tempest et al., (1991) *Biotechnology* 9, 266-273.

#### Antagonists and Agonists—Assays and Molecules

Polypeptides and polynucleotides of the invention may also be used to assess the binding of small molecule substrates and ligands in, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. These substrates and ligands may be natural substrates and ligands or may be structural or functional mimetics. See, e.g., Coligan et al., *Current Protocols in Immunology* 1(2): Chapter 5 (1991).

The screening methods may simply measure the binding of a candidate compound to the polypeptide or polynucleotide, or to cells or membranes bearing the polypeptide or polynucleotide, or a fusion protein of the polypeptide by means of a label directly or indirectly associated with the candidate compound. Alternatively, the screening method may involve competition with a labeled competitor. Further, these screening methods may test whether the candidate compound results in a signal generated by activation or inhibition of the polypeptide or polynucleotide, using detection systems appropriate to the cells comprising the polypeptide or polynucleotide. Inhibitors of activation are generally assayed in the presence of a known agonist and the effect on activation by the agonist by the presence of the candidate compound is observed. Constitutively active polypeptide and/or constitutively expressed polypeptides and polynucleotides may be employed in screening methods for inverse agonists or inhibitors, in the absence of an agonist or inhibitor, by testing whether the candidate compound results in inhibition of activation of the polypeptide or polynucleotide, as the case may be. Further, the screening methods may simply comprise the steps of mixing a candidate compound with a solution containing a polypeptide or polynucleotide of the present invention, to form a mixture, measuring BASB006 polypeptide and/or polynucleotide activity in the mixture, and comparing the BASB006 polypeptide and/or polynucleotide activity of the mixture to a standard. Fusion proteins, such as those made from Fc portion and BASB006 polypeptide, as hereinbefore described, can also be used for high-throughput screening assays to identify antagonists of the polypeptide of the present invention, as well as of phylogenetically and/or functionally related polypeptides (see D. Bennett et al., *J Mol Recognition*, 8:52-58 (1995); and K. Johanson et al., *J Biol Chem*, 270(16):9459-9471 (1995)).

The polynucleotides, polypeptides and antibodies that bind to and/or interact with a polypeptide of the present invention may also be used to configure screening methods for detecting the effect of added compounds on the production of mRNA and/or polypeptide in cells. For example, an ELISA assay may be constructed for measuring secreted or cell associated levels of polypeptide using monoclonal and polyclonal antibodies by standard methods known in the art. This can be used to discover agents which may inhibit or enhance the

production of polypeptide (also called antagonist or agonist, respectively) from suitably manipulated cells or tissues.

The invention also provides a method of screening compounds to identify those which enhance (agonist) or block (antagonist) the action of BASB006 polypeptides or polynucleotides, particularly those compounds that are bacteriostatic and/or bactericidal. The method of screening may involve high-throughput techniques. For example, to screen for agonists or antagonists, a synthetic reaction mix, a cellular compartment, such as a membrane, cell envelope or cell wall, or a preparation of any thereof, comprising BASB006 polypeptide and a labeled substrate or ligand of such polypeptide is incubated in the absence or the presence of a candidate molecule that may be a BASB006 agonist or antagonist. The ability of the candidate molecule to agonize or antagonize the BASB006 polypeptide is reflected in decreased binding of the labeled ligand or decreased production of product from such substrate. Molecules that bind gratuitously, i.e., without inducing the effects of BASB006 polypeptide are most likely to be good antagonists. Molecules that bind well and, as the case may be, increase the rate of product production from substrate, increase signal transduction, or increase chemical channel activity are agonists. Detection of the rate or level of, as the case may be, production of product from substrate, signal transduction, or chemical channel activity may be enhanced by using a reporter system. Reporter systems that may be useful in this regard include but are not limited to colorimetric, labeled substrate converted into product, a reporter gene that is responsive to changes in BASB006 polynucleotide or polypeptide activity, and binding assays known in the art.

Another example of an assay for BASB006 agonists is a competitive assay that combines BASB006 and a potential agonist with BASB006-binding molecules, recombinant BASB006 binding molecules, natural substrates or ligands, or substrate or ligand mimetics, under appropriate conditions for a competitive inhibition assay. BASB006 can be labeled, such as by radioactivity or a colorimetric compound, such that the number of BASB006 molecules bound to a binding molecule or converted to product can be determined accurately to assess the effectiveness of the potential antagonist.

Potential antagonists include, among others, small organic molecules, peptides, polypeptides and antibodies that bind to a polynucleotide and/or polypeptide of the invention and thereby inhibit or extinguish its activity or expression. Potential antagonists also may be small organic molecules, a peptide, a polypeptide such as a closely related protein or antibody that binds the same sites on a binding molecule, such as a binding molecule, without inducing BASB006-induced activities, thereby preventing the action or expression of BASB006 polypeptides and/or polynucleotides by excluding BASB006 polypeptides and/or polynucleotides from binding.

Potential antagonists include a small molecule that binds to and occupies the binding site of the polypeptide thereby preventing binding to cellular binding molecules, such that normal biological activity is prevented. Examples of small molecules include but are not limited to small organic molecules, peptides or peptide-like molecules. Other potential antagonists include antisense molecules (see Okano, *J. Neurochem.* 56: 560 (1991); *OLIGODEOXYNUCLEOTIDES AS ANTISENSE INHIBITORS OF GENE EXPRESSION*, CRC Press, Boca Raton, Fla. (1988), for a description of these molecules). Preferred potential antagonists include compounds related to and variants of BASB006.

In a further aspect, the present invention relates to genetically engineered soluble fusion proteins comprising a

polypeptide of the present invention, or a fragment thereof, and various portions of the constant regions of heavy or light chains of immunoglobulins of various subclasses (IgG, IgM, IgA, IgE). Preferred as an immunoglobulin is the constant part of the heavy chain of human IgG, particularly IgG1, where fusion takes place at the hinge region. In a particular embodiment, the Fc part can be removed simply by incorporation of a cleavage sequence which can be cleaved with blood clotting factor Xa. Furthermore, this invention relates to processes for the preparation of these fusion proteins by genetic engineering, and to the use thereof for drug screening, diagnosis and therapy. A further aspect of the invention also relates to polynucleotides encoding such fusion proteins. Examples of fusion protein technology can be found in International Patent Application Nos. WO94/29458 and WO94/22914.

Each of the polynucleotide sequences provided herein may be used in the discovery and development of antibacterial compounds. The encoded protein, upon expression, can be used as a target for the screening of antibacterial drugs. Additionally, the polynucleotide sequences encoding the amino terminal regions of the encoded protein or Shine-Delgarno or other translation facilitating sequences of the respective mRNA can be used to construct antisense sequences to control the expression of the coding sequence of interest.

The invention also provides the use of the polypeptide, polynucleotide, agonist or antagonist of the invention to interfere with the initial physical interaction between a pathogen or pathogens and a eukaryotic, preferably mammalian, host responsible for sequelae of infection. In particular, the molecules of the invention may be used: in the prevention of adhesion of bacteria, in particular gram positive and/or gram negative bacteria, to eukaryotic, preferably mammalian, extracellular matrix proteins on in-dwelling devices or to extracellular matrix proteins in wounds; to block bacterial adhesion between eukaryotic, preferably mammalian, extracellular matrix proteins and bacterial BASB006 proteins that mediate tissue damage and/or; to block the normal progression of pathogenesis in infections initiated other than by the implantation of in-dwelling devices or by other surgical techniques.

In accordance with yet another aspect of the invention, there are provided BASB006 agonists and antagonists, preferably bacteriostatic or bactericidal agonists and antagonists.

The antagonists and agonists of the invention may be employed, for instance, to prevent, inhibit and/or treat diseases.

In a further aspect, the present invention relates to mimotopes of the polypeptide of the invention. A mimotope is a peptide sequence, sufficiently similar to the native peptide (sequentially or structurally), which is capable of being recognised by antibodies which recognise the native peptide; or is capable of raising antibodies which recognise the native peptide when coupled to a suitable carrier.

Peptide mimotopes may be designed for a particular purpose by addition, deletion or substitution of elected amino acids. Thus, the peptides may be modified for the purposes of ease of conjugation to a protein carrier. For example, it may be desirable for some chemical conjugation methods to include a terminal cysteine. In addition it may be desirable for peptides conjugated to a protein carrier to include a hydrophobic terminus distal from the conjugated terminus of the peptide, such that the free unconjugated end of the peptide remains associated with the surface of the carrier protein. Thereby presenting the peptide in a conformation which most closely resembles that of the peptide as found in the context of the whole native molecule. For example, the peptides may be

altered to have an N-terminal cysteine and a C-terminal hydrophobic amidated tail. Alternatively, the addition or substitution of a D-stereoisomer form of one or more of the amino acids may be performed to create a beneficial derivative, for example to enhance stability of the peptide.

Alternatively, peptide mimotopes may be identified using antibodies which are capable themselves of binding to the polypeptides of the present invention using techniques such as phage display technology (EP 0 552 267 B1). This technique generates a large number of peptide sequences which mimic the structure of the native peptides and are, therefore, capable of binding to anti-native peptide antibodies, but may not necessarily themselves share significant sequence homology to the native polypeptide.

#### Vaccines

Another aspect of the invention relates to a method for inducing an immunological response in an individual, particularly a mammal, preferably humans, which comprises inoculating the individual with BASB006 polynucleotide and/or polypeptide, or a fragment or variant thereof, adequate to produce antibody and/or T cell immune response to protect said individual from infection, particularly bacterial infection and most particularly *Neisseria meningitidis* infection. Also provided are methods whereby such immunological response slows bacterial replication. Yet another aspect of the invention relates to a method of inducing immunological response in an individual which comprises delivering to such individual a nucleic acid vector, sequence or ribozyme to direct expression of BASB006 polynucleotide and/or polypeptide, or a fragment or a variant thereof, for expressing BASB006 polynucleotide and/or polypeptide, or a fragment or a variant thereof in vivo in order to induce an immunological response, such as, to produce antibody and/or T cell immune response, including, for example, cytokine-producing T cells or cytotoxic T cells, to protect said individual, preferably a human, from disease, whether that disease is already established within the individual or not. One example of administering the gene is by accelerating it into the desired cells as a coating on particles or otherwise. Such nucleic acid vector may comprise DNA, RNA, a ribozyme, a modified nucleic acid, a DNA/RNA hybrid, a DNA-protein complex or an RNA-protein complex.

A further aspect of the invention relates to an immunological composition that when introduced into an individual, preferably a human, capable of having induced within it an immunological response, induces an immunological response in such individual to a BASB006 polynucleotide and/or polypeptide encoded therefrom, wherein the composition comprises a recombinant BASB006 polynucleotide and/or polypeptide encoded therefrom and/or comprises DNA and/or RNA which encodes and expresses an antigen of said BASB006 polynucleotide, polypeptide encoded therefrom, or other polypeptide of the invention. The immunological response may be used therapeutically or prophylactically and may take the form of antibody immunity and/or cellular immunity, such as cellular immunity arising from CTL or CD4+ T cells.

A BASB006 polypeptide or a fragment thereof may be fused with co-protein or chemical moiety which may or may not by itself produce antibodies, but which is capable of stabilizing the first protein and producing a fused or modified protein which will have antigenic and/or immunogenic properties, and preferably protective properties. Thus fused recombinant protein, preferably further comprises an antigenic co-protein, such as lipoprotein D from *Haemophilus influenzae*, Glutathione-S-transferase (GST) or beta-galac-



tosidase, or any other relatively large co-protein which solubilizes the protein and facilitates production and purification thereof. Moreover, the co-protein may act as an adjuvant in the sense of providing a generalized stimulation of the immune system of the organism receiving the protein. The co-protein may be attached to either the amino- or carboxy-terminus of the first protein.

Provided by this invention are compositions, particularly vaccine compositions, and methods comprising the polypeptides and/or polynucleotides of the invention and immunostimulatory DNA sequences, such as those described in Sato, Y. et al. Science 273: 352 (1996).

Also, provided by this invention are methods using the described polynucleotide or particular fragments thereof, which have been shown to encode non-variable regions of bacterial cell surface proteins, in polynucleotide constructs used in such genetic immunization experiments in animal models of infection with *Neisseria meningitidis*. Such experiments will be particularly useful for identifying protein epitopes able to provoke a prophylactic or therapeutic immune response. It is believed that this approach will allow for the subsequent preparation of monoclonal antibodies of particular value, derived from the requisite organ of the animal successfully resisting or clearing infection, for the development of prophylactic agents or therapeutic treatments of bacterial infection, particularly *Neisseria meningitidis* infection, in mammals, particularly humans.

The invention also includes a vaccine formulation which comprises an immunogenic recombinant polypeptide and/or polynucleotide of the invention together with a suitable carrier, such as a pharmaceutically acceptable carrier. Since the polypeptides and polynucleotides may be broken down in the stomach, each is preferably administered parenterally, including, for example, administration that is subcutaneous, intramuscular, intravenous, or intradermal. Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostatic compounds and solutes which render the formulation isotonic with the bodily fluid, preferably the blood, of the individual; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use.

The vaccine formulation of the invention may also include adjuvant systems for enhancing the immunogenicity of the formulation. Preferably the adjuvant system raises preferentially a TH1 type of response.

An immune response may be broadly distinguished into two extreme categories, being a humoral or cell mediated immune responses (traditionally characterised by antibody and cellular effector mechanisms of protection respectively). These categories of response have been termed TH1-type responses (cell-mediated response), and TH2-type immune responses (humoral response).

Extreme TH1-type immune responses may be characterised by the generation of antigen specific, haplotype restricted cytotoxic T lymphocytes, and natural killer cell responses. In mice TH1-type responses are often characterised by the generation of antibodies of the IgG2a subtype, whilst in the human these correspond to IgG1 type antibodies. TH2-type immune responses are characterised by the generation of a broad range of immunoglobulin isotypes including in mice IgG1, IgA, and IgM.

It can be considered that the driving force behind the development of these two types of immune responses are cytokines. High levels of TH1-type cytokines tend to favour the induction of cell mediated immune responses to the given antigen, whilst high levels of TH2-type cytokines tend to favour the induction of humoral immune responses to the antigen.

The distinction of TH1 and TH2-type immune responses is not absolute. In reality an individual will support an immune response which is described as being predominantly TH1 or predominantly TH2. However, it is often convenient to consider the families of cytokines in terms of that described in murine CD4 +ve T cell clones by Mosmann and Coffman (Mosmann, T. R. and Coffman, R. L. (1989) *TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annual Review of Immunology*, 7, p145-173). Traditionally, TH1-type responses are associated with the production of the TNF- $\gamma$  and IL-2 cytokines by T-lymphocytes. Other cytokines often directly associated with the induction of TH1-type immune responses are not produced by T-cells, such as IL-12. In contrast, TH2-type responses are associated with the secretion of IL-4, IL-5, IL-6 and IL-13.

It is known that certain vaccine adjuvants are particularly suited to the stimulation of either TH1 or TH2-type cytokine responses. Traditionally the best indicators of the TH1:TH2 balance of the immune response after a vaccination or infection includes direct measurement of the production of TH1 or TH2 cytokines by T lymphocytes in vitro after restimulation with antigen, and/or the measurement of the IgG1:IgG2a ratio of antigen specific antibody responses.

Thus, a TH1-type adjuvant is one which preferentially stimulates isolated T-cell populations to produce high levels of TH1-type cytokines when re-stimulated with antigen in vitro, and promotes development of both CD8+ cytotoxic T lymphocytes and antigen specific immunoglobulin responses associated with TH1-type isotype.

Adjuvants which are capable of preferential stimulation of the TH1 cell response are described in International Patent Application No. WO 94/00153 and WO 95/17209.

3 De-O-acylated monophosphoryl lipid A (3D-MPL) is one such adjuvant. This is known from GB 2220211 (Ribi). Chemically it is a mixture of 3 De-O-acylated monophosphoryl lipid A with 4, 5 or 6 acylated chains and is manufactured by Ribi Immunochem, Montana. A preferred form of 3 De-O-acylated monophosphoryl lipid A is disclosed in European Patent 0 689 454 B1 (SmithKline Beecham Biologicals SA).

Preferably, the particles of 3D-MPL are small enough to be sterile filtered through a 0.22 micron membrane (European Patent number 0 689 454). 3D-MPL will be present in the range of 10  $\mu$ g-100  $\mu$ g preferably 25-50  $\mu$ g per dose wherein the antigen will typically be present in a range 2-50  $\mu$ g per dose.

Another preferred adjuvant comprises QS21, an Hplc purified non-toxic fraction derived from the bark of *Quillaja Saponaria Molina*. Optionally this may be admixed with 3 De-O-acylated monophosphoryl lipid A (3D-MPL), optionally together with a carrier.

The method of production of QS21 is disclosed in U.S. Pat. No. 5,057,540.

Non-reactogenic adjuvant formulations containing QS21 have been described previously (WO 96/33739). Such formulations comprising QS21 and cholesterol have been shown to be successful TH1 stimulating adjuvants when formulated together with an antigen.

Further adjuvants which are preferential stimulators of TH1 cell response include immunomodulatory oligonucleotides, for example unmethylated CpG sequences as disclosed in WO 96/02555.

Combinations of different TH1 stimulating adjuvants, such as those mentioned hereinabove, are also contemplated as providing an adjuvant which is a preferential stimulator of TH1 cell response. For example, QS21 can be formulated together with 3D-MPL. The ratio of QS21: 3D-MPL will typically be in the order of 1:10 to 10:1; preferably 1:5 to 5:1 and often substantially 1:1. The preferred range for optimal synergy is 2.5:1 to 1:1 3D-MPL: QS21.

Preferably a carrier is also present in the vaccine composition according to the invention. The carrier may be an oil in water emulsion, or an aluminium salt, such as aluminium phosphate or aluminium hydroxide.

A preferred oil-in-water emulsion comprises a metabolizable oil, such as squalene, alpha tocopherol and TWEEN® 80 ((80)-sorbitan mono-9-octadecenoate poly(oxy-1,2-ethanediyl); Uniquema/ICI). In a particularly preferred aspect the antigens in the vaccine composition according to the invention are combined with QS21 and 3D-MPL in such an emulsion. Additionally the oil-in-water emulsion may contain span 85 and/or lecithin and/or tricaprilyn.

Typically for human administration QS21 and 3D-MPL will be present in a vaccine in the range of 1 µg-200 µg, such as 10 µg-100 µg, preferably 10 µg-50 µg per dose. Typically the oil in water will comprise from 2 to 10% squalene, from 2 to 10% alpha tocopherol and from 0.3 to 3% TWEEN® 80 ((80)-sorbitan mono-9-octadecenoate poly(oxy-1,2-ethanediyl); Uniquema/ICI). Preferably the ratio of squalene: alpha tocopherol is equal to or less than 1, as this provides a more stable emulsion. Span 85 may also be present at a level of 1%. In some cases it may be advantageous that the vaccines of the present invention will further contain a stabiliser.

Non-toxic oil-in-water emulsions preferably contain a non-toxic oil, e.g. squalene or squalene, an emulsifier, e.g., TWEEN® 80 ((80)-sorbitan mono-9-octadecenoate poly(oxy-1,2-ethanediyl); Uniquema/ICI), in an aqueous carrier. The aqueous carrier may be, for example, phosphate buffered saline.

A particularly potent adjuvant formulation involving QS21, 3D-MPL and tocopherol in an oil in water emulsion is described in WO 95/17210.

The present invention also provides a polyvalent vaccine composition comprising a vaccine formulation of the invention in combination with other antigens, in particular antigens useful for treating cancers, autoimmune diseases and related conditions. Such a polyvalent vaccine composition may include a TH-1 inducing adjuvant as hereinbefore described.

While the invention has been described with reference to certain BASB006 polypeptides and polynucleotides, it is to be understood that this covers fragments of the naturally occurring polypeptides and polynucleotides, and similar polypeptides and polynucleotides with additions, deletions or substitutions which do not substantially affect the immunogenic properties of the recombinant polypeptides or polynucleotides.

The antigen can also be delivered in the form of whole bacteria (dead or alive) or as subcellular fractions, these possibilities do include *N. meningitidis* itself.

#### Compositions, Kits and Administration

In a further aspect of the invention there are provided compositions comprising a BASB006 polynucleotide and/or a BASB006 polypeptide for administration to a cell or to a multicellular organism.

The invention also relates to compositions comprising a polynucleotide and/or a polypeptide discussed herein or their agonists or antagonists. The polypeptides and polynucleotides of the invention may be employed in combination with a non-sterile or sterile carrier or carriers for use with cells, tissues or organisms, such as a pharmaceutical carrier suitable for administration to an individual. Such compositions comprise, for instance, a media additive or a therapeutically effective amount of a polypeptide and/or polynucleotide of the invention and a pharmaceutically acceptable carrier or excipient. Such carriers may include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol and combinations thereof. The formulation should suit the mode of administration. The invention further relates to diagnostic and pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention.

Polypeptides, polynucleotides and other compounds of the invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

The pharmaceutical compositions may be administered in any effective, convenient manner including, for instance, administration by topical, oral, anal, vaginal, intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal or intradermal routes among others.

In therapy or as a prophylactic, the active agent may be administered to an individual as an injectable composition, for example as a sterile aqueous dispersion, preferably isotonic.

In a further aspect, the present invention provides for pharmaceutical compositions comprising a therapeutically effective amount of a polypeptide and/or polynucleotide, such as the soluble form of a polypeptide and/or polynucleotide of the present invention, agonist or antagonist peptide or small molecule compound, in combination with a pharmaceutically acceptable carrier or excipient. Such carriers include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The invention further relates to pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention. Polypeptides, polynucleotides and other compounds of the present invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

The composition will be adapted to the route of administration, for instance by a systemic or an oral route. Preferred forms of systemic administration include injection, typically by intravenous injection. Other injection routes, such as subcutaneous, intramuscular, or intraperitoneal, can be used. Alternative means for systemic administration include trans-mucosal and transdermal administration using penetrants such as bile salts or fusidic acids or other detergents. In addition, if a polypeptide or other compounds of the present invention can be formulated in an enteric or an encapsulated formulation, oral administration may also be possible. Administration of these compounds may also be topical and/or localized, in the form of salves, pastes, gels, solutions, powders and the like.

For administration to mammals, and particularly humans, it is expected that the daily dosage level of the active agent will be from 0.01 mg/kg to 10 mg/kg, typically around 1 mg/kg. The physician in any event will determine the actual dosage which will be most suitable for an individual and will vary with the age, weight and response of the particular individual. The above dosages are exemplary of the average case.



There can, of course, be individual instances where higher or lower dosage ranges are merited, and such are within the scope of this invention.

The dosage range required depends on the choice of peptide, the route of administration, the nature of the formulation, the nature of the subject's condition, and the judgment of the attending practitioner. Suitable dosages, however, are in the range of 0.1-100 µg/kg of subject.

A vaccine composition is conveniently in injectable form. Conventional adjuvants may be employed to enhance the immune response. A suitable unit dose for vaccination is 0.5-5 microgram/kg of antigen, and such dose is preferably administered 1-3 times and with an interval of 1-3 weeks. With the indicated dose range, no adverse toxicological effects will be observed with the compounds of the invention which would preclude their administration to suitable individuals.

Wide variations in the needed dosage, however, are to be expected in view of the variety of compounds available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, as is well understood in the art.

#### Sequence Databases, Sequences in a Tangible Medium, and Algorithms

Polynucleotide and polypeptide sequences form a valuable information resource with which to determine their 2- and 3-dimensional structures as well as to identify further sequences of similar homology. These approaches are most easily facilitated by storing the sequence in a computer readable medium and then using the stored data in a known macromolecular structure program or to search a sequence database using well known searching tools, such as the GCG program package.

Also provided by the invention are methods for the analysis of character sequences or strings, particularly genetic sequences or encoded protein sequences. Preferred methods of sequence analysis include, for example, methods of sequence homology analysis, such as identity and similarity analysis, DNA, RNA and protein structure analysis, sequence assembly, cladistic analysis, sequence motif analysis, open reading frame determination, nucleic acid base calling, codon usage analysis, nucleic acid base trimming, and sequencing chromatogram peak analysis.

A computer based method is provided for performing homology identification. This method comprises the steps of: providing a first polynucleotide sequence comprising the sequence of a polynucleotide of the invention in a computer readable medium; and comparing said first polynucleotide sequence to at least one second polynucleotide or polypeptide sequence to identify homology.

A computer based method is also provided for performing homology identification, said method comprising the steps of: providing a first polypeptide sequence comprising the sequence of a polypeptide of the invention in a computer readable medium; and comparing said first polypeptide sequence to at least one second polynucleotide or polypeptide sequence to identify homology.

All publications and references, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference in their entirety as if each individual publication or reference were specifically and individually indicated to be incorporated by reference herein as being fully set forth. Any patent application to which this

application claims priority is also incorporated by reference herein in its entirety in the manner described above for publications and references.

#### DEFINITIONS

"Identity," as known in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as the case may be, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. "Identity" can be readily calculated by known methods, including but not limited to those described in (*Computational Molecular Biology*, Lesk, A. M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D. W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data*, Part I, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heine, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., *SIAM J. Applied Math.*, 48: 1073 (1988). Methods to determine identity are designed to give the largest match between the sequences tested. Moreover, methods to determine identity are codified in publicly available computer programs. Computer program methods to determine identity between two sequences include, but are not limited to, the GAP program in the GCG program package (Devereux, J., et al., *Nucleic Acids Research* 12(1): 387 (1984)), BLASTP, BLASTN (Altschul, S. F. et al., *J. Molec. Biol.* 215: 403-410 (1990), and FASTA (Pearson and Lipman Proc. Natl. Acad. Sci. USA 85: 2444-2448 (1988)). The BLAST family of programs is publicly available from NCBI and other sources (*BLAST Manual*, Altschul, S., et al., NCBI NLM NIH Bethesda, Md. 20894; Altschul, S., et al., *J. Mol. Biol.* 215: 403-410 (1990)). The well known Smith Waterman algorithm may also be used to determine identity.

Parameters for polypeptide sequence comparison include the following:

Algorithm: Needleman and Wunsch, *J. Mol Biol.* 48: 443-453 (1970)

Comparison matrix: BLOSSUM62 from Henikoff and Henikoff,

Proc. Natl. Acad. Sci. USA. 89:10915-10919 (1992)

Gap Penalty: 8

Gap Length Penalty: 2

A program useful with these parameters is publicly available as the "gap" program from Genetics Computer Group, Madison Wis. The aforementioned parameters are the default parameters for peptide comparisons (along with no penalty for end gaps).

Parameters for polynucleotide comparison include the following:

Algorithm: Needleman and Wunsch, *J. Mol Biol.* 48: 443-453 (1970)

Comparison matrix: matches=+10, mismatch=0

Gap Penalty: 50

Gap Length Penalty: 3

Available as: The "gap" program from Genetics Computer Group, Madison Wis. These are the default parameters for nucleic acid comparisons.

A preferred meaning for "identity" for polynucleotides and polypeptides, as the case may be, are provided in (1) and (2) below.

(1) Polynucleotide embodiments further include an isolated polynucleotide comprising a polynucleotide sequence having at least a 50, 60, 70, 80, 85, 90, 95, 97 or 100% identity to the reference sequence of SEQ ID NO:1, wherein said polynucleotide sequence may be identical to the reference sequence of SEQ ID NO:1 or may include up to a certain integer number of nucleotide alterations as compared to the reference sequence, wherein said alterations are selected from the group consisting of at least one nucleotide deletion, substitution, including transition and transversion, or insertion, and wherein said alterations may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among the nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence, and wherein said number of nucleotide alterations is determined by multiplying the total number of nucleotides in SEQ ID NO:1 by the integer defining the percent identity divided by 100 and then subtracting that product from said total number of nucleotides in SEQ ID NO:1, or:

$$n_n \leq x_n - (x_n \cdot y),$$

wherein  $n_n$  is the number of nucleotide alterations,  $x_n$  is the total number of nucleotides in SEQ ID NO:1,  $y$  is 0.50 for 50%, 0.60 for 60%, 0.70 for 70%, 0.80 for 80%, 0.85 for 85%, 0.90 for 90%, 0.95 for 95%, 0.97 for 97% or 1.00 for 100%, and  $\cdot$  is the symbol for the multiplication operator, and wherein any non-integer product of  $x_n$  and  $y$  is rounded down to the nearest integer prior to subtracting it from  $x_n$ . Alterations of a polynucleotide sequence encoding the polypeptide of SEQ ID NO:2 may create nonsense, missense or frameshift mutations in this coding sequence and thereby alter the polypeptide encoded by the polynucleotide following such alterations.

By way of example, a polynucleotide sequence of the present invention may be identical to the reference sequence of SEQ ID NO:1, that is it may be 100% identical, or it may include up to a certain integer number of nucleic acid alterations as compared to the reference sequence such that the percent identity is less than 100% identity. Such alterations are selected from the group consisting of at least one nucleic acid deletion, substitution, including transition and transversion, or insertion, and wherein said alterations may occur at the 5' or 3' terminal positions of the reference polynucleotide sequence or anywhere between those terminal positions, interspersed either individually among the nucleic acids in the reference sequence or in one or more contiguous groups within the reference sequence. The number of nucleic acid alterations for a given percent identity is determined by multiplying the total number of nucleic acids in SEQ ID NO:1 by the integer defining the percent identity divided by 100 and then subtracting that product from said total number of nucleic acids in SEQ ID NO:1, or:

$$n_n \leq x_n - (x_n \cdot y),$$

wherein  $n_n$  is the number of nucleic acid alterations,  $x_n$  is the total number of nucleic acids in SEQ ID NO:1,  $y$  is, for instance 0.70 for 70%, 0.80 for 80%, 0.85 for 85% etc.,  $\cdot$  is the symbol for the multiplication operator, and wherein any non-integer product of  $x_n$  and  $y$  is rounded down to the nearest integer prior to subtracting it from  $x_n$ .

(2) Polypeptide embodiments further include an isolated polypeptide comprising a polypeptide having at least a 50, 60, 70, 80, 85, 90, 95, 97 or 100% identity to a polypeptide reference sequence of SEQ ID NO:2, wherein said polypep-

ptide sequence may be identical to the reference sequence of SEQ ID NO:2 or may include up to a certain integer number of amino acid alterations as compared to the reference sequence, wherein said alterations are selected from the group consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion, and wherein said alterations may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence, and wherein said number of amino acid alterations is determined by multiplying the total number of amino acids in SEQ ID NO:2 by the integer defining the percent identity divided by 100 and then subtracting that product from said total number of amino acids in SEQ ID NO:2, or:

$$n_a \leq x_a - (x_a \cdot y),$$

wherein  $n_a$  is the number of amino acid alterations,  $x_a$  is the total number of amino acids in SEQ ID NO:2,  $y$  is 0.50 for 50%, 0.60 for 60%, 0.70 for 70%, 0.80 for 80%, 0.85 for 85%, 0.90 for 90%, 0.95 for 95%, 0.97 for 97% or 1.00 for 100%, and  $\cdot$  is the symbol for the multiplication operator, and wherein any non-integer product of  $x_a$  and  $y$  is rounded down to the nearest integer prior to subtracting it from  $x_a$ .

By way of example, a polypeptide sequence of the present invention may be identical to the reference sequence of SEQ ID NO:2, that is it may be 100% identical, or it may include up to a certain integer number of amino acid alterations as compared to the reference sequence such that the percent identity is less than 100% identity. Such alterations are selected from the group consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion, and wherein said alterations may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence. The number of amino acid alterations for a given % identity is determined by multiplying the total number of amino acids in SEQ ID NO:2 by the integer defining the percent identity divided by 100 and then subtracting that product from said total number of amino acids in SEQ ID NO:2, or:

$$n_a \leq x_a - (x_a \cdot y),$$

wherein  $n_a$  is the number of amino acid alterations,  $x_a$  is the total number of amino acids in SEQ ID NO:2,  $y$  is, for instance 0.70 for 70%, 0.80 for 80%, 0.85 for 85% etc., and  $\cdot$  is the symbol for the multiplication operator, and wherein any non-integer product of  $x_a$  and  $y$  is rounded down to the nearest integer prior to subtracting it from  $x_a$ .

"Individual(s)," when used herein with reference to an organism, means a multicellular eukaryote, including, but not limited to a metazoan, a mammal, an ovid, a bovid, a simian, a primate, and a human.

"Isolated" means altered "by the hand of man" from its natural state, i.e., if it occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living organism is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated," as the term is employed herein. Moreover, a polynucleotide or polypeptide that is introduced into an organism by transformation, genetic manipulation or

by any other recombinant method is "isolated" even if it is still present in said organism, which organism may be living or non-living.

"Polynucleotide(s)" generally refers to any polyribonucleotide or polydeoxyribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA including single and double-stranded regions.

"Variant" refers to a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

"Disease(s)" means any disease caused by or related to infection by a bacteria, including, for example, upper respiratory tract infection, invasive bacterial diseases, such as bacteremia and meningitis.

### EXAMPLES

The examples below are carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. The examples are illustrative, but do not limit the invention.

#### Example 1

##### Discovery and Confirmatory DNA Sequencing of the BASB006 Gene from Two *N. meningitidis* Strains

A: BASB006 in *N. meningitidis* Serogroup B Strain ATCC13090.

The BASB006 gene disclosed in SEQ ID NO:1 was first discovered in the Incyte PathoSeq database containing unfinished genomic DNA sequences of the *N. meningitidis* strain ATCC13090. The translation of the BASB006 polynucleotide sequence, shown in SEQ ID NO:2, showed significant similarity (56% identity in a 1455 amino acids overlap) to the Hap protein of *Haemophilus influenzae*, a polypeptide reported previously to function as an adhesin. The sequence of the BASB006 gene was confirmed experimentally. For this purpose, genomic DNA was extracted from  $10^{10}$  cells of the *N. meningitidis* cells (strain ATCC 13090) using the QIAGEN genomic DNA extraction kit (Qiagen GmbH), and 1 µg of this material was submitted to Polymerase Chain Reaction DNA amplification using primers Hap01 (5'-GGG GGC TAG CAA AAC AAC CGA CAA ACG GAC AAC C-3') [SEQ ID NO:5] and Hap02 (5'-GGG GAA GCT TCC AGC GGT AGC GGT

AGC CTA ATT TGA TGC C-3') [SEQ ID NO:6]. This PCR product was gel-purified and subjected to DNA sequencing using the Big Dye Cycle Sequencing kit (Perkin-Elmer) and an ABI 373A/PRISM DNA sequencer. DNA sequencing was performed on both strands with a redundancy of 2 and the full-length sequence was assembled using the SeqMan program from the DNASTAR Lasergene software package. The resulting DNA sequence turned out to be 100% identical to SEQ ID NO:1.

B: BASB006 in *N. meningitidis* Serogroup B Strain H44/76.

The sequence of the BASB006 gene was also determined in another *N. meningitidis* serogroup B strain, the strain H44/76. For this purpose, genomic DNA was extracted from the *N. meningitidis* strain H44/76 using the experimental conditions presented in Example 1. This material (1 µg) was then submitted to Polymerase Chain Reaction DNA amplification using primers Hap01 and Hap02 specific for the BASB006 gene. A 4389 bp DNA fragment was obtained, digested by the *NheI*/*HindIII* restriction endonucleases and inserted into the corresponding sites of the pET-24b cloning/expression vector (Novagen) using standard molecular biology techniques (Molecular Cloning, a Laboratory Manual, Second Edition, Eds: Sambrook, Fritsch & Maniatis, Cold Spring Harbor press 1989). Recombinant pET-24b/BASB006 was then submitted to DNA sequencing using the Big Dyes kit (Applied biosystems) and analyzed on a ABI 373A DNA sequencer in the conditions described by the supplier. As a result, the polynucleotide and deduced polypeptide sequences, referred to as SEQ ID NO:3 and SEQ ID NO:4 respectively, were obtained. Using the PILEUP program from the GCG package, an alignment of the polynucleotide sequences of SEQ ID NO:1 and 3 was performed, and is displayed in FIG. 1; their level of identity amounts to 97.8%, as determined by the GAP program. Using the same PILEUP program, an alignment of the polypeptide sequences of SEQ ID NO:2 and 4 was performed, and is displayed in FIG. 2; their level of identity amounts to 97.0%, as determined by the GAP program. Taken together, these data indicate strong sequence conservation of the BASB006 gene among the two *N. meningitidis* serogroup B strains.

#### Example 2

##### Expression and Purification of Recombinant BASB006 Protein in *Escherichia coli*

The construction of the pET-24b/BASB006 cloning/expression vector was described in Example 1B. This vector harbours the BASB006 gene isolated from the strain H44/76 in fusion with a stretch of 6 histidine residues, placed under the control of the strong bacteriophage T7 gene 10 promoter. For expression study, this vector was introduced into the *Escherichia coli* strain BL21 DE3 (Novagen), in which, the gene for the T7 polymerase is placed under the control of the isopropyl-beta-D thiogalactoside (IPTG)-regulatable lac promoter. Liquid cultures (100 ml) of the BL21 DE-3 [pET-24b/BASB006] *E. coli* recombinant strain were grown at 37° C. under agitation until the optical density at 600 nm (OD600) reached 0.6. At that time-point, IPTG was added at a final concentration of 1 mM and the culture was grown for 4 additional hours. The culture was then centrifuged at 10,000 rpm and the pellet was frozen at -20° C. for at least 10 hours. After thawing, the pellet was resuspended during 30 min at 25° C. in buffer A (6M guanidine hydrochloride, 0.1M NaH<sub>2</sub>PO<sub>4</sub>, 0.01M Tris, pH 8.0), passed three-times through a needle and clarified by centrifugation (20000 rpm, 15 min).

The sample was then loaded at a flow-rate of 1 ml/min on a Ni<sup>2+</sup>-loaded Hitrap column (Pharmacia Biotech). After passage of the flowthrough, the column was washed successively with 40 ml of buffer B (8M Urea, 0.1M NaH<sub>2</sub>PO<sub>4</sub>, 0.01M Tris, pH 8.0), 40 ml of buffer C (8M Urea, 0.1M NaH<sub>2</sub>PO<sub>4</sub>, 0.01M Tris, pH 6.3). The recombinant protein BASB006/His6 was then eluted from the column with 30 ml of buffer C (8M Urea, 0.1M NaH<sub>2</sub>PO<sub>4</sub>, 0.01M Tris, pH 6.3) containing 500 mM of imidazole and 3 ml-size fractions were collected. In FIG. 3, substantially purified proteins were separated on a 4-20% gradient polyacrylamide gel under PAGE-SDS conditions and stained with Coomassie Blue R250. The sample loaded on the gel corresponded to protein fractions enriched (more than 80%) in BASB006 (lane 1 and 2) and a molecular weight marker (MW). As shown in FIG. 3, a highly enriched (Purity estimated to more than 90% pure in coomassie staining) BASB006/His6 protein, migrating at 170 kDa (estimated relative molecular mass), was eluted from the column. This polypeptide was reactive against a mouse monoclonal antibody raised against the 5-histidine motif. Taken together, these data indicate that the BASB006 gene can be expressed and purified under a recombinant form (BASB006/His6) in *E. coli*.

## Example 3

#### Immunization of Mice with Recombinant BASB006 and Recognition of the BASB006 Polypeptide on Different *N. meningitidis* Serogroup B Strains by Western Blotting

Partially purified recombinant BASB006 expressed in *E. coli* has been injected three times in BALB/C mice on days 0, 14 and 28 (10 animals/group). Animals were injected by the subcutaneous route with 5 µg of antigen r formulated in SB62 emulsion containing 5 µg MPL and 1 µg QS21 per dose. Mice were bled on days 29 (15 days Post II) and 35 (6 days Post III) in order to detect specific anti-BASB006 antibodies. Specific anti-BASB006 antibodies were measured on pooled sera (from 10 mice/group) by western-blotting on six different *Neisseria meningitidis* serogroup B strains (FIG. 4).

The six different *Neisseria meningitidis* B strains are: H44/76 (B:15:P1.7, 16, lineage ET-5), M97 250987 (B:4:P1.15), BZ10 (B:2b:P1.2, lineage A4), BZ198 (B:NT\*: -, lineage 3), and EG328 (B:NT\*, lineage ST-18), and on partially purified recombinant BASB006 protein (mixed with two other candidate antigens). (\*: NT: Not Typed).

Briefly, 15 µl (>10<sup>8</sup> cells/lane) of each sample treated with sample buffer (10 min at 95° C.) are put into a SDS-PAGE gradient gel (Tris-glycine 4-20%, Novex, code no. EC60252). Electrophoretic migration occurs at 125 volts for 90 min. Afterwards, proteins are transferred to a nitrocellulose sheet (0.45 µm, Bio-rad code no. 162-0114) at 100 volts for 1 hour using a Bio-rad Trans-blot system (code no. 170-3930). The filter was blocked with PBS-0.050% TWEEN® 20 overnight at room temperature, before incubation with the mice sera containing the anti-BASB006 antibodies. These sera are diluted 100 times in PBS-0.050% TWEEN® 20 and incubated on the nitrocellulose sheet for two hours at room temperature with gentle shaking, using a mini-blotter system (Miniprotean, Bio-rad code no. 170-4017). After three repeated washing steps in PBS-0.050% TWEEN® 20 ((20)-sorbitan mono-9-octadecenoate poly(oxy-1,2-ethanediyl); Uniquema/ICI) for 5 min., the nitrocellulose sheet is incubated at room temperature for 1 hour under gentle shaking with the appropriate conjugate (biotinylated anti-mouse Ig antibodies from sheep, Amersham code no. RPN 1001)

diluted at 1/500 in the same washing buffer. The membrane is washed three times as previously, and incubated for 30 min. with agitation using the streptavidin-peroxidase complex (Amersham code no. 1051) diluted at 1/1000 in the washing buffer. After the last three repeated washing steps, the revelation occurs during the 20 min. incubation time in a 50 ml solution containing 30 mg 4-chloro-1-naphthol (Sigma), 10 ml methanol, 40 ml PBS, and 30 µl of H<sub>2</sub>O<sub>2</sub>. The staining is stopped while washing the membrane several times in distilled water.

FIG. 4 shows recognition of the native BASB006 protein from several *Neisseria meningitidis* serogroup B strains by sera from immunized mice. Results illustrated in FIG. 4 show that all strains tested present a band around 95-100 kD (see arrow), which is probably the extracellular part of the BASB006 protein (after cleavage of the intact molecule into two pieces, which is known to occur in the *H. influenzae* Hap protein). This means that the BASB006 protein is probably expressed in most of the *Neisseria meningitidis* serogroup B strains. All other bands could be antibodies directed against degradation products, or against cross-reacting antigens between *E. coli* and *Neisseria meningitidis* B strains, as the preparation used for immunization still contained *E. coli* contaminants.

## Example 4

#### Presence of Anti-BASB006 Antibodies in Sera From Human Convalescent Patients

In this test, human convalescent sera were tested by western-blotting for recognition of the purified recombinant BASB006 protein.

5 µg of partially purified BASB006 protein mixed with two other *Neisseria meningitidis* serogroup B proteins are put into a SDS-PAGE gradient gel (4-20%, Novex, code no. EC60252) for electrophoretic migration. Proteins are transferred to nitrocellulose sheet (0.4 µm, Bio-rad code no. 162-0114) at 100 volts for 1 hour using a Bio-rad Trans-blot system (code no. 170-3930). Afterwards, the filter is blocked with PBS-0.05% TWEEN® 20 ((20)-sorbitan mono-9-octadecenoate poly(oxy-1,2-ethanediyl); Uniquema/ICI) overnight at room temperature, before incubation with the human sera. These sera are diluted 100 times in PBS-0.05% TWEEN® 20, and incubated on the nitrocellulose sheet for two hours at room temperature with gentle shaking, using a mini-blotter system (Miniprotean, Bio-rad code no. 170-4017). After three repeated washing steps in PBS-0.05% TWEEN® 20 ((20)-sorbitan mono-9-octadecenoate poly(oxy-1,2-ethanediyl); Uniquema/ICI) for 5 min., the nitrocellulose sheet is incubated at room temperature for 1 hour under gentle shaking with the appropriate conjugate (biotinylated anti-human Ig antibodies, from sheep, Amersham code no. RPN1003) diluted at 1/500 in the same washing buffer. The membrane is washed three times as previously, and incubated for 30 min. with agitation using the streptavidin-peroxidase complex (Amersham code no. 1051) diluted at 1/1000 in the washing buffer. After the last three repeated washing steps, the revelation occurs during the 20 min. incubation time in a 50 ml solution containing 30 mg 4-chloro-1-naphthol (Sigma), 10 ml methanol, 40 ml of ultra-pure water, and 30 µl of H<sub>2</sub>O<sub>2</sub>. The staining is stopped while washing the membrane several times in distilled water.

Results illustrated in FIG. 5 (Part B) show that all convalescent sera react against the intact BASB006 protein at around 160 kD, while 3 out of 7 convalescent sera are reacting against the possible processed BASB006 protein (+/-95-100

kD). The BASB006 bands are clearly visible at these two molecular weights (95-100 and 160 kD). In part A of the western-blot, it can be seen that mice sera (mixture of specific antibodies against three different Ag candidates) recognize the intact recombinant BASB006 protein at the same molecular weight, while at the lower MW, it is more difficult to discriminate which of the two bands around 95 kD is related to the processed BASB006 protein.

#### Deposited Materials

A deposit containing a *Neisseria meningitidis* Serogroup B strain has been deposited with the American Type Culture Collection (herein "ATCC") on Jun. 22, 1997 and assigned deposit number 13090. The deposit was described as *Neisseria meningitidis* (Albrecht and Ghon) and is a freeze-dried, 1.5-2.9 kb insert library constructed from *N. meningitidis* isolate. The deposit is described in Int. Bull. Bacteriol. Nomencl. Taxon. 8: 1-15 (1958).

The *Neisseria meningitidis* strain deposit is referred to herein as "the deposited strain" or as "the DNA of the deposited strain."

The deposited strain contains the full length BASB006 gene. The sequence of the polynucleotides contained in the deposited strain, as well as the amino acid sequence of any polypeptide encoded thereby, are controlling in the event of any conflict with any description of sequences herein.

The deposit of the deposited strain has been made under the terms of the Budapest Treaty on the International Recognition of the Deposit of Micro-organisms for Purposes of Patent Procedure. The strain will be irrevocably and without restriction or condition released to the public upon the issuance of a patent. The deposited strain is provided merely as convenience to those of skill in the art and is not an admission that a deposit is required for enablement, such as that required under 35 U.S.C. §112.

#### SEQUENCE LISTING

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Phe	Phe	Ser	Gly	Arg	Pro	Thr	Pro	His	Ala	Tyr	Asn	His	Leu	Gly	Ser
			645						650					655	
Gly	Trp	Ser	Lys	Met	Glu	Gly	Ile	Pro	Gln	Gly	Glu	Ile	Val	Trp	Asp
			660					665					670		
Asn	Asp	Trp	Ile	Asn	Arg	Thr	Phe	Lys	Ala	Glu	Asn	Phe	His	Ile	Gln



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675					680					685					
Gly	Gly	Gln	Ala	Val	Ile	Ser	Arg	Asn	Val	Ala	Lys	Val	Glu	Gly	Asp
690					695					700					
Trp	His	Leu	Ser	Asn	His	Ala	Gln	Ala	Val	Phe	Gly	Val	Ala	Pro	His
705					710					715					720
Gln	Ser	His	Thr	Ile	Cys	Thr	Arg	Ser	Asp	Trp	Thr	Gly	Leu	Thr	Asn
				725					730					735	
Cys	Val	Glu	Lys	Thr	Ile	Thr	Asp	Asp	Lys	Val	Ile	Ala	Ser	Leu	Thr
			740					745					750		
Lys	Thr	Asp	Ile	Ser	Gly	Asn	Val	Ser	Leu	Ala	Asp	His	Ala	His	Leu
		755					760					765			
Asn	Leu	Thr	Gly	Leu	Ala	Thr	Leu	Asn	Gly	Asn	Leu	Ser	Ala	Asn	Gly
		770					775					780			
Asp	Thr	Arg	Tyr	Thr	Val	Ser	His	Asn	Ala	Thr	Gln	Asn	Gly	Asp	Leu
785							790					795			800
Ser	Leu	Val	Gly	Asn	Ala	Gln	Ala	Thr	Phe	Asn	Gln	Ala	Thr	Leu	Asn
				805					810					815	
Gly	Asn	Thr	Ser	Ala	Ser	Gly	Asn	Ala	Ser	Phe	Asn	Leu	Ser	Asn	Asn
			820					825					830		
Ala	Val	Gln	Asn	Gly	Ser	Leu	Thr	Leu	Ser	Gly	Asn	Ala	Lys	Ala	Asn
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Val	Ser	His	Ser	Ala	Leu	Asn	Gly	Asn	Val	Ser	Leu	Ala	Asp	Lys	Ala
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865							870					875			880
Lys	Asp	Thr	Ala	Leu	His	Leu	Lys	Asp	Ser	Glu	Trp	Thr	Leu	Pro	Ser
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Gly	Thr	Glu	Leu	Gly	Asn	Leu	Asn	Leu	Asp	Asn	Ala	Thr	Ile	Thr	Leu
			900					905					910		
Asn	Ser	Ala	Tyr	Arg	His	Asp	Ala	Ala	Gly	Ala	Gln	Thr	Gly	Ser	Ala
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Thr	Asp	Ala	Pro	Arg	Arg	Arg	Ser	Arg	Arg	Ser	Leu	Leu	Ser	Val	Thr
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Pro	Pro	Ala	Ser	Ala	Glu	Ser	His	Phe	Asn	Thr	Leu	Thr	Val	Asn	Gly
945							950					955			960
Lys	Leu	Asn	Gly	Gln	Gly	Thr	Phe	Arg	Phe	Met	Ser	Glu	Leu	Phe	Gly
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Tyr	Arg	Ser	Asp	Lys	Leu	Lys	Leu	Ala	Glu	Ser	Ser	Glu	Gly	Thr	Tyr
			980					985					990		
Thr	Leu	Ala	Val	Asn	Asn	Thr	Gly	Asn	Glu	Pro	Val	Ser	Leu	Asp	Gln
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Gln	Leu	Ile	Arg	Lys	Asp	Gly	Glu	Phe	Arg	Leu	His	Asn	Pro	Val	Lys
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Glu	Gln	Glu	Leu	Ser	Asp	Lys	Leu	Gly	Lys	Ala	Glu	Ala	Lys	Lys	Gln
			1060					1065					1070		
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Ala Gly Gly Glu Asn Val Gly Ile Met Gln Ala Glu Glu Glu Lys Lys  
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Arg Val Gln Ala Asp Lys Asp Thr Ala Leu Ala Lys Gln Arg Glu Gly  
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Arg Asp Leu Pro Gln Pro Gln Pro Gln Pro Gln Pro Gln Arg  
 1155 1160 1165

Asp Leu Ile Ser Arg Tyr Ala Asn Ser Gly Leu Ser Glu Phe Ser Ala  
 1170 1175 1180

Thr Leu Asn Ser Val Phe Ala Val Gln Asp Glu Leu Asp Arg Val Phe  
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Ala Glu Asp Arg Arg Asn Ala Val Trp Thr Ser Gly Ile Arg Asp Thr  
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Lys His Tyr Arg Ser Gln Asp Phe Arg Ala Tyr Arg Gln Gln Thr Asp  
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Leu Arg Gln Ile Gly Met Gln Lys Asn Leu Gly Ser Gly Arg Val Gly  
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Ile Leu Phe Ser His Asn Arg Thr Glu Asn Thr Phe Asp Asp Gly Ile  
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Gly Asn Ser Ala Arg Leu Ala His Gly Ala Val Phe Gly Gln Tyr Gly  
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Ile Gly Arg Phe Asp Ile Gly Ile Ser Thr Gly Ala Gly Phe Ser Ser  
 1285 1290 1295

Gly Ser Leu Ser Asp Asp Ile Gly Ser Lys Ile Arg Arg Arg Val Leu  
 1300 1305 1310

His Tyr Gly Ile Gln Ala Arg Tyr Arg Ala Gly Phe Gly Gly Phe Gly  
 1315 1320 1325

Ile Glu Pro His Ile Gly Ala Thr Arg Tyr Phe Val Gln Lys Ala Asp  
 1330 1335 1340

Tyr Arg Tyr Glu Asn Val Asn Ile Ala Thr Pro Gly Leu Ala Phe Asn  
 1345 1350 1355 1360

Arg Tyr Arg Ala Gly Ile Lys Ala Asp Tyr Ser Phe Lys Pro Ala Gln  
 1365 1370 1375

His Ile Ser Ile Thr Pro Tyr Leu Ser Leu Ser Tyr Thr Asp Ala Ala  
 1380 1385 1390

Ser Gly Lys Val Arg Thr Arg Val Asn Thr Ala Val Leu Ala Gln Asp  
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Phe Gly Lys Thr Arg Ser Ala Glu Trp Gly Val Asn Ala Glu Ile Lys  
 1410 1415 1420

Gly Phe Thr Leu Ser Leu His Ala Ala Ala Lys Gly Pro Gln Leu  
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Glu Ala Gln His Ser Ala Gly Ile Lys Leu Gly Tyr Arg Trp  
 1445 1450

<210> SEQ ID NO 3  
 <211> LENGTH: 4374  
 <212> TYPE: DNA  
 <213> ORGANISM: Bacteria

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aacgttgatt	ttggtgcgga	aggaagaaat	cccgatcaac	atcgttttac	ttataaaatt	420
gtgaaacgga	ataattataa	agcagggact	aaaggccatc	cttatgggtg	cgattatcat	480
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<210> SEQ ID NO 4
<211> LENGTH: 1457
<212> TYPE: PRT
<213> ORGANISM: Bacteria

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<400> SEQUENCE: 4

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Ser Phe Gly Ile Leu Pro Gln Ala Trp Ala Gly His Thr Tyr Phe Gly
 35             40             45

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 85 90 95  
 Ser Arg Asn Gly Val Ala Ala Leu Val Gly Asp Gln Tyr Ile Val Ser  
 100 105 110  
 Val Ala His Asn Gly Gly Tyr Asn Asn Val Asp Phe Gly Ala Glu Gly  
 115 120 125  
 Arg Asn Pro Asp Gln His Arg Phe Thr Tyr Lys Ile Val Lys Arg Asn  
 130 135 140  
 Asn Tyr Lys Ala Gly Thr Lys Gly His Pro Tyr Gly Gly Asp Tyr His  
 145 150 155 160  
 Met Pro Arg Leu His Lys Phe Val Thr Asp Ala Glu Pro Val Glu Met  
 165 170 175  
 Thr Ser Tyr Met Asp Gly Arg Lys Tyr Ile Asp Gln Asn Asn Tyr Pro  
 180 185 190  
 Asp Arg Val Arg Ile Gly Ala Gly Arg Gln Tyr Trp Arg Ser Asp Glu  
 195 200 205  
 Asp Glu Pro Asn Asn Arg Glu Ser Ser Tyr His Ile Ala Ser Ala Tyr  
 210 215 220  
 Ser Trp Leu Val Gly Gly Asn Thr Phe Ala Gln Asn Gly Ser Gly Gly  
 225 230 235 240  
 Gly Thr Val Asn Leu Gly Ser Glu Lys Ile Lys His Ser Pro Tyr Gly  
 245 250 255  
 Phe Leu Pro Thr Gly Gly Ser Phe Gly Asp Ser Gly Ser Pro Met Phe  
 260 265 270  
 Ile Tyr Asp Ala Gln Lys Gln Lys Trp Leu Ile Asn Gly Val Leu Gln  
 275 280 285  
 Thr Gly Asn Pro Tyr Ile Gly Lys Ser Asn Gly Phe Gln Leu Val Arg  
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 Lys Asp Trp Phe Tyr Asp Glu Ile Phe Ala Gly Asp Thr His Ser Val  
 305 310 315 320  
 Phe Tyr Glu Pro Arg Gln Asn Gly Lys Tyr Ser Phe Asn Asp Asp Asn  
 325 330 335  
 Asn Gly Thr Gly Lys Ile Asn Ala Lys His Glu His Asn Ser Leu Pro  
 340 345 350  
 Asn Arg Leu Lys Thr Arg Thr Val Gln Leu Phe Asn Val Ser Leu Ser  
 355 360 365  
 Glu Thr Ala Arg Glu Pro Val Tyr His Ala Ala Gly Gly Val Asn Ser  
 370 375 380  
 Tyr Arg Pro Arg Leu Asn Asn Gly Glu Asn Ile Ser Phe Ile Asp Glu  
 385 390 395 400  
 Gly Lys Gly Glu Leu Ile Leu Thr Ser Asn Ile Asn Gln Gly Ala Gly  
 405 410 415  
 Gly Leu Tyr Phe Gln Gly Asp Phe Thr Val Ser Pro Glu Asn Asn Glu  
 420 425 430  
 Thr Trp Gln Gly Ala Gly Val His Ile Ser Glu Asp Ser Thr Val Thr  
 435 440 445  
 Trp Lys Val Asn Gly Val Ala Asn Asp Arg Leu Ser Lys Ile Gly Lys  
 450 455 460

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Val	Gly	Asp	Gly	Thr	Val	Ile	Leu	Asp	Gln	Gln	Ala	Asp	Asp	Lys	Gly
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Lys	Lys	Gln	Ala	Phe	Ser	Glu	Ile	Gly	Leu	Val	Ser	Gly	Arg	Gly	Thr
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Val	Gln	Leu	Asn	Ala	Asp	Asn	Gln	Phe	Asn	Pro	Asp	Lys	Leu	Tyr	Phe
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Gly	Phe	Arg	Gly	Gly	Arg	Leu	Asp	Leu	Asn	Gly	His	Ser	Leu	Ser	Phe
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His	Arg	Ile	Gln	Asn	Thr	Asp	Glu	Gly	Ala	Met	Ile	Val	Asn	His	Asn
545					550					555					560
Gln	Asp	Lys	Glu	Ser	Thr	Val	Thr	Ile	Thr	Gly	Asn	Lys	Asp	Ile	Ala
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Thr	Thr	Gly	Asn	Asn	Asn	Ser	Leu	Asp	Ser	Lys	Lys	Glu	Ile	Ala	Tyr
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		595					600					605			
Asn	Leu	Val	Tyr	Gln	Pro	Ala	Ala	Glu	Asp	Arg	Thr	Leu	Leu	Leu	Ser
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Gly	Gly	Thr	Asn	Leu	Asn	Gly	Asn	Ile	Thr	Gln	Thr	Asn	Gly	Lys	Leu
625				630						635					640
Phe	Phe	Ser	Gly	Arg	Pro	Thr	Pro	His	Ala	Tyr	Asn	His	Leu	Asn	Asp
				645					650					655	
His	Trp	Ser	Gln	Lys	Glu	Gly	Ile	Pro	Arg	Gly	Glu	Ile	Val	Trp	Asp
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Trp	His	Leu	Ser	Asn	His	Ala	Gln	Ala	Val	Phe	Gly	Val	Ala	Pro	His
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Gln	Ser	His	Thr	Ile	Cys	Thr	Arg	Ser	Asp	Trp	Thr	Gly	Leu	Thr	Asn
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Cys	Val	Glu	Lys	Thr	Ile	Thr	Asp	Asp	Lys	Val	Ile	Ala	Ser	Leu	Thr
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Lys	Thr	Asp	Ile	Ser	Gly	Asn	Val	Asp	Leu	Ala	Asp	His	Ala	His	Leu
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Asp	Thr	Arg	Tyr	Thr	Val	Ser	His	Asn	Ala	Thr	Gln	Asn	Gly	Asn	Leu
785					790					795					800
Ser	Leu	Val	Gly	Asn	Ala	Gln	Ala	Thr	Phe	Asn	Gln	Ala	Thr	Leu	Asn
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885					890					895					
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Asn	Ser	Ala	Tyr	Arg	His	Asp	Ala	Ala	Gly	Ala	Gln	Thr	Gly	Ser	Ala
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Thr	Asp	Ala	Pro	Arg	Arg	Ser	Arg	Arg	Ser	Arg	Arg	Ser	Leu	Leu	
		930					935					940			
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Val	Asn	Gly	Lys	Leu	Asn	Gly	Gln	Gly	Thr	Phe	Arg	Phe	Met	Ser	Glu
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Trp	Arg	Tyr	Gln	Leu	Ile	Arg	Lys	Asp	Gly	Glu	Phe	Arg	Leu	His	Asn
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Glu	Lys	Lys	Arg	Val	Gln	Ala	Asp	Lys	Asp	Thr	Ala	Leu	Ala	Lys	Gln
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Arg	Glu	Ala	Glu	Thr	Arg	Pro	Ala	Thr	Thr	Ala	Phe	Pro	Arg	Ala	Arg
		1140						1145					1150		
Arg	Ala	Arg	Arg	Asp	Leu	Pro	Gln	Leu	Gln	Pro	Gln	Pro	Gln	Pro	Gln
		1155					1160					1165			
Pro	Gln	Arg	Asp	Leu	Ile	Ser	Arg	Tyr	Ala	Asn	Ser	Gly	Leu	Ser	Glu
		1170					1175					1180			
Phe	Ser	Ala	Thr	Leu	Asn	Ser	Val	Phe	Ala	Val	Gln	Asp	Glu	Leu	Asp
		1185					1190					1195			1200
Arg	Val	Phe	Ala	Glu	Glu	Arg	Arg	Asn	Ala	Val	Trp	Thr	Ser	Gly	Ile
			1205					1210					1215		
Arg	Asp	Thr	Lys	His	Tyr	Arg	Ser	Gln	Asp	Phe	Arg	Ala	Tyr	Arg	Gln
		1220						1225					1230		
Gln	Thr	Asp	Leu	Arg	Gln	Ile	Gly	Met	Gln	Lys	Asn	Leu	Gly	Ser	Gly
		1235					1240					1245			
Arg	Val	Gly	Ile	Leu	Phe	Ser	His	Asn	Arg	Thr	Glu	Asn	Thr	Phe	Asp
		1250					1255					1260			
Asp	Gly	Ile	Gly	Asn	Ser	Ala	Arg	Leu	Ala	His	Gly	Ala	Val	Phe	Gly
		1265					1270					1275			1280
Gln	Tyr	Gly	Ile	Asp	Arg	Phe	Tyr	Ile	Gly	Ile	Ser	Ala	Gly	Ala	Gly
			1285					1290					1295		
Phe	Ser	Ser	Gly	Ser	Leu	Ser	Asp	Gly	Ile	Gly	Gly	Lys	Ile	Arg	Arg
			1300					1305					1310		

-continued

Arg Val Leu His Tyr Gly Ile Gln Ala Arg Tyr Arg Ala Gly Phe Gly  
 1315 1320 1325

Gly Phe Gly Ile Glu Pro His Ile Gly Ala Thr Arg Tyr Phe Val Gln  
 1330 1335 1340

Lys Ala Asp Tyr Arg Tyr Glu Asn Val Asn Ile Ala Thr Pro Gly Leu  
 1345 1350 1355 1360

Ala Phe Asn Arg Tyr Arg Ala Gly Ile Lys Ala Asp Tyr Ser Phe Lys  
 1365 1370 1375

Pro Ala Gln His Ile Ser Ile Thr Pro Tyr Leu Ser Leu Ser Tyr Thr  
 1380 1385 1390

Asp Ala Ala Ser Gly Lys Val Arg Thr Arg Val Asn Thr Ala Val Leu  
 1395 1400 1405

Ala Gln Asp Phe Gly Lys Thr Arg Ser Ala Glu Trp Gly Val Asn Ala  
 1410 1415 1420

Glu Ile Lys Gly Phe Thr Leu Ser Leu His Ala Ala Ala Lys Gly  
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Pro Gln Leu Glu Ala Gln His Ser Ala Gly Ile Lys Leu Gly Tyr Arg  
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Trp

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34

<210> SEQ ID NO 6  
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40

What is claimed is:

1. An isolated polynucleotide comprising a first polynucleotide sequence or the full complement of the first polynucleotide sequence, wherein the first polynucleotide sequence encodes a polypeptide selected from the group consisting of SEQ ID NO:2 or 4.
2. The isolated polynucleotide of claim 1, wherein the isolated polynucleotide comprises the first polynucleotide sequence.
3. The isolated polynucleotide of claim 2, wherein the first polynucleotide sequence encodes the polypeptide consisting of SEQ ID NO:2.
4. The isolated polynucleotide of claim 3, wherein the isolated polynucleotide consists of the first polynucleotide sequence.
5. The isolated polynucleotide of claim 2, wherein the first polynucleotide sequence encodes the polypeptide consisting of SEQ ID NO:4.
6. The isolated polynucleotide of claim 5, wherein the isolated polynucleotide consists of the first polynucleotide sequence.
7. An expression vector comprising the isolated polynucleotide of claim 1.
8. A host cell comprising the expression vector of claim 7.
9. An immunogenic composition comprising the expression vector of claim 7 and a pharmaceutically acceptable carrier.
10. The immunogenic composition of claim 9, further comprising an adjuvant.
11. An isolated polynucleotide comprising a first polynucleotide or the full complement of the first polynucleotide sequence, wherein the first polynucleotide sequence is selected from the group consisting of SEQ ID NO:1 or 3.
12. The isolated polynucleotide of claim 11, wherein the isolated polynucleotide comprises the first polynucleotide sequence.



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13. The isolated polynucleotide of claim 12, wherein the first polynucleotide sequence consists of SEQ ID NO:1.

14. The isolated polynucleotide of claim 13, wherein the isolated polynucleotide consists of the first polynucleotide sequence.

15. The isolated polynucleotide of claim 12, wherein the first polynucleotide sequence consists of SEQ ID NO:3.

16. The isolated polynucleotide of claim 15, wherein the isolated polynucleotide consists of the first polynucleotide sequence.

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17. An expression vector comprising the isolated polynucleotide of claim 11.

18. A host cell comprising the expression vector of claim 17.

19. An immunogenic composition comprising the expression vector of claim 17 and a pharmaceutically acceptable carrier.

20. The immunogenic composition of claim 19, further comprising an adjuvant.

\* \* \* \* \*

## Exhibit B

cent of his annual workload. *Id.* at 15:12-16. Moreover, according to Geiseler, the need to review proposed legislation and FDIC regulations, tasks that had also been performed by the plaintiff, had diminished. *Id.* at 16:14-17:22.

In the final analysis, the Court is not persuaded that the plaintiff has provided sufficient evidence to persuade a reasonable jury that "the defendant intentionally discriminated against [her]...." *Burdine*, 450 U.S. at 253, 101 S.Ct. 1089. Accordingly, the defendant is entitled to summary judgment on the plaintiff's Title VII race discrimination claim.

#### IV. Conclusion

For the foregoing reasons, the Court concludes that the plaintiff has failed to show that the defendant's legitimate, non-discriminatory reason for her reassignment was a pretext for discrimination and therefore the defendant's motion for summary judgment must be granted.

**SO ORDERED** on this 30th day of September, 2008.<sup>10</sup>



WYETH, et al., Plaintiffs,

v.

Jon W. DUDAS, Under Secretary of  
Commerce for Intellectual Property  
and Director of U.S. Patent and  
Trademark Office, Defendant.

Civil Action No. 07-1492 (JR).

United States District Court,  
District of Columbia.

Sept. 30, 2008.

**Background:** Patent owners brought action challenging method used by Patent

10. An Order consistent with this Court's ruling is being issued simultaneously with this

and Trademark Office (PTO) to calculate extensions of patent terms for certain specified kinds of PTO delay.

**Holding:** The District Court, James Robertson, J., held that extension of patent term based on PTO delay could exceed three years.

Ordered accordingly.

#### 1. Patents ⇌ 133

Patent and Trademark Office's (PTO) interpretation of statute providing extensions of patent terms for certain specified kinds of PTO delay was not entitled to *Chevron* deference. 35 U.S.C.A. § 154(b)(2)(C)(iii), (b)(3)(A).

#### 2. Patents ⇌ 133

Extension of patent term for any administrative delay by Patent and Trademark Office (PTO) in issuing patent more than three years after filing date did not necessarily overlap with extensions provided as result of PTO's failure to comply with enumerated statutory deadlines, and thus statutory prohibition against double-counting did not preclude patent applicants from claiming extensions in excess of three years. 35 U.S.C.A. § 154(b).

David O. Bickart, Kaye Scholer LLP,  
Washington, DC, Patricia A. Carson, Kaye  
Scholer LLP, New York, NY, for Plain-  
tiffs.

Fred Elmore Haynes, U.S. Attorney's  
Office, Washington, DC, for Defendant.

opinion.

## MEMORANDUM OPINION

JAMES ROBERTSON, District Judge.

Plaintiffs here take issue with the interpretation that the United States Patent and Trademark Office (PTO) has imposed upon 35 U.S.C. § 154, the statute that prescribes patent terms. Section 154(a)(2) establishes a term of 20 years from the day on which a successful patent application is first filed. Because the clock begins to run on this filing date, and not on the day the patent is actually granted, some of the effective term of a patent is consumed by the time it takes to prosecute the application. To mitigate the damage that bureaucracy can do to inventors, the statute grants extensions of patent terms for certain specified kinds of PTO delay, 35 U.S.C. § 154(b)(1)(A), and, regardless of the reason, whenever the patent prosecution takes more than three years, 35 U.S.C. § 154(b)(1)(B). Recognizing that the protection provided by these separate guarantees might overlap, Congress has forbidden double-counting: "To the extent that periods of delay attributable to grounds specified in paragraph (1) overlap, the period of any adjustment granted under this subsection shall not exceed the actual number of days the issuance of the patent was delayed." 35 U.S.C. § 154(b)(2)(A). Plaintiffs claim that the PTO has misconstrued or misapplied this provision, and that the PTO is denying them a portion of the term Congress has provided for the protection of their intellectual property rights.

## Statutory Scheme

Until 1994, patent terms were 17 years from the date of issuance. *See* 35 U.S.C. § 154 (1992) ("Every patent shall contain ... a grant ... for the term of seventeen years ... of the right to exclude others

from making, using, or selling the invention throughout the United States...."). In 1994, in order to comply with treaty obligations under the General Agreement on Tariffs and Trade (GATT), the statute was amended to provide a 20-year term from the date on which the application is first filed. *See* Pub.L. No. 103-465, § 532, 108 Stat. 4809, 4984 (1994). In 1999, concerned that extended prosecution delays could deny inventors substantial portions of their effective patent terms under the new regime, Congress enacted the American Inventors Protection Act, a portion of which—referred to as the Patent Term Guarantee Act of 1999—provided for the adjustments that are at issue in this case. Pub.L. No. 106-113, §§ 4401-4402, 113 Stat. 1501, 1501A-557 (1999).

As currently codified, 35 U.S.C. § 154(b) provides three guarantees of patent term, two of which are at issue here. The first is found in subsection (b)(1)(A), the "[g]uarantee of prompt Patent and Trademark Office response." It provides a one-day extension of patent term for every day that issuance of a patent is delayed by a failure of the PTO to comply with various enumerated statutory deadlines: fourteen months for a first office action; four months to respond to a reply; four months to issue a patent after the fee is paid; and the like. *See* 35 U.S.C. § 154(b)(1)(A)(i)-(iv). Periods of delay that fit under this provision are called "A delays" or "A periods." The second provision is the "[g]uarantee of no more than 3-year application pendency." Under this provision, a one-day term extension is granted for every day greater than three years after the filing date that it takes for the patent to issue, regardless of whether the delay is the fault of the PTO.<sup>1</sup> *See* 35 U.S.C.

1. Certain reasons for exceeding the three-year pendency period are excluded, *see* 35 U.S.C.

§ 154(b)(1)(b)(i)-(iii), as are periods attribut-

§ 154(b)(1)(B). The period that begins after the three-year window has closed is referred to as the "B delay" or the "B period". ("C delays," delays resulting from interferences, secrecy orders, and appeals, are similarly treated but were not involved in the patent applications underlying this suit.)

The extensions granted for A, B, and C delays are subject to the following limitation:

(A) In general.—To the extent that periods of delay attributable to grounds specified in paragraph (1) overlap, the period of any adjustment granted under this subsection shall not exceed the actual number of days the issuance of the patent was delayed.

35 U.S.C. § 154(b)(2)(A). This provision is manifestly intended to prevent double-counting of periods of delay, but understanding that intent does not answer the question of what is double-counting and what is not. Proper interpretation of this proscription against windfall extensions requires an assessment of what it means for "periods of delay" to "overlap."

The PTO, pursuant to its power under 35 U.S.C. § 154(b)(3)(A) to "prescribe regulations establishing procedures for the application for and determination of patent term adjustments," has issued final rules and an "explanation" of the rules, setting forth its authoritative construction of the double-counting provision. The rules that the PTO has promulgated essentially parrot the statutory text, *see* 37 C.F.R. § 1.703(f), and so the real interpretive act is found in something the PTO calls its Explanation of 37 CFR 1.703(f) and of the United States Patent and Trademark Office Interpretation of 35 U.S.C. § 154(b)(2)(A), which was published on

June 21, 2004, at 69 Fed.Reg. 34238. Here, the PTO "explained" that:

the Office has consistently taken the position that if an application is entitled to an adjustment under the three-year pendency provision of 35 U.S.C. § 154(b)(1)(B), *the entire period during which the application was pending before the Office* (except for periods excluded under 35 U.S.C. § 154(b)(1)(B)(i)-(iii)), and not just the period beginning three years after the actual filing date of the application, *is the relevant period under 35 U.S.C. § 154(b)(1)(B) in determining whether periods of delay "overlap" under 35 U.S.C. 154(b)(2)(A).*

69 Fed.Reg. 34238 (2004) (emphasis added). In short, the PTO's view is that any administrative delay under § 154(b)(1)(A) overlaps any 3-year maximum pendency delay under § 154(b)(1)(B): the applicant gets credit for "A delay" or for "B delay," whichever is larger, but never A + B.

In the plaintiffs' submission, this interpretation does not square with the language of the statute. They argue that the "A period" and "B period" overlap only if they occur on the same calendar day or days. Consider this example, proffered by plaintiff: A patent application is filed on 1/1/02. The patent issues on 1/1/08, six years later. In that six-year period are two "A periods," each one year long: (1) the 14-month deadline for first office action is 3/1/03, but the first office action does not occur until 3/1/04, one year late; (2) the 4-month deadline for patent issuance after payment of the issuance fee is 1/1/07, but the patent does not issue until 1/1/08, another year of delay attributable to the PTO. According to plaintiff, the "B period" begins running on 1/1/05, three years after the patent application was filed, and ends three years later, with the issuance of the patent on 1/1/08. In this

able to the applicant's own delay. *See* 35

U.S.C. § 154(b)(2)(C).

example, then, the first "A period" does not overlap the "B period," because it occurs in 2003–04, not in 2005–07. The second "A period," which covers 365 of the same days covered by the "B period," does overlap. Thus, in plaintiff's submission, this patent holder is entitled to four years of adjustment (one year of "A period" delay + three years of "B period" delay). But in the PTO's view, since "the entire period during which the application was pending before the office" is considered to be "B period" for purposes of identifying "overlap," the patent holder gets only three years of adjustment.

#### *Chevron* Deference

We must first decide whether the PTO's interpretation is entitled to deference under *Chevron v. NRDC*, 467 U.S. 837, 104 S.Ct. 2778, 81 L.Ed.2d 694 (1984). No, the plaintiffs argue, because, under the Supreme Court's holdings in *Gonzales v. Oregon*, 546 U.S. 243, 126 S.Ct. 904, 163 L.Ed.2d 748 (2006), and *United States v. Mead Corp.*, 533 U.S. 218, 121 S.Ct. 2164, 150 L.Ed.2d 292 (2001), Congress has not "delegated authority to the agency generally to make rules carrying the force of law," and in any case the interpretation at issue here was not promulgated pursuant to any such authority. See *Gonzales*, 546 U.S. at 255–56, 126 S.Ct. 904, citing *Mead*, 533 U.S. at 226–27, 121 S.Ct. 2164. Since at least 1996, the Federal Circuit has held that the PTO is not afforded *Chevron* deference because it does not have the authority to issue substantive rules, only procedural regulations regarding the conduct of proceedings before the agency. See *Merck & Co. v. Kessler*, 80 F.3d 1543, 1549–50 (Fed.Cir.1996).

[1] Here, as in *Merck*, the authority of the PTO is limited to prescribing "regulations establishing procedures for the application for and determination of patent term adjustments under this subsection."

35 U.S.C. § 154(b)(3)(A) (emphasis added). Indeed, a comparison of this rulemaking authority with the authority conferred for a different purpose in the immediately preceding section of the statute makes it clear that the PTO's authority to interpret the overlap provision is quite limited. In 35 U.S.C. § 154(b)(2)(C)(iii) the PTO is given the power to "prescribe regulations establishing the *circumstances that constitute* a failure of an applicant to engage in reasonable efforts to conclude processing or examination of an application" (emphasis added)—that is, the power to elaborate on the meaning of a particular statutory term. No such power is granted under § 154(b)(3)(A). *Chevron* deference does not apply to the interpretation at issue here.

#### Statutory Construction

*Chevron* would not save the PTO's interpretation, however, because it cannot be reconciled with the plain text of the statute. If the statutory text is not ambiguous enough to permit the construction that the agency urges, that construction fails at *Chevron*'s "step one," without regard to whether it is a reasonable attempt to reach a result that Congress might have intended. See, e.g., *MCI v. AT & T*, 512 U.S. 218, 229, 114 S.Ct. 2223, 129 L.Ed.2d 182 (1994) ("[A]n agency's interpretation of a statute is not entitled to deference when it goes beyond the meaning that the statute can bear.").

The operative question under 35 U.S.C. § 154(b)(2)(A) is whether "periods of delay attributable to grounds specified in paragraph (1) overlap." The only way that periods of time can "overlap" is if they occur on the same day. If an "A delay" occurs on one calendar day and a "B delay" occurs on another, they do not overlap, and § 154(b)(2)(A) does not limit the extension to one day. Recognizing this,

the PTO defends its interpretation as essentially running the "period of delay" under subsection (B) from the filing date of the patent application, such that a period of "B delay" *always overlaps* with any periods of "A delay" for the purposes of applying § 154(b)(2)(A).

[2] The problem with the PTO's construction is that it considers the application *delayed* under § 154(b)(1)(B) during the period *before it has been delayed*. That construction cannot be squared with the language of § 154(b)(1)(B), which applies "if the issue of an original patent is *delayed* due to the failure of the United States Patent and Trademark Office to issue a patent within 3 years." (Emphasis added.) "B delay" begins when the PTO has failed to issue a patent within three years, not before.

The PTO's interpretation appears to be driven by Congress's admonition that any term extension "not exceed the actual number of days the issuance of the patent was delayed," and by the PTO's view that "A delays" during the first three years of an applications' pendency inevitably lead to "B delays" in later years. Thus, as the PTO sees it, if plaintiffs' construction is adopted, one cause of delay will be counted twice: once because the PTO has failed to meet an administrative deadline, and again because that failure has pushed back the entire processing of the application into the "B period." Indeed, in the example set forth above, plaintiffs' calendar-day construction does result in a total effective patent term of 18 years under the (B) guarantee, so that—again from the PTO's viewpoint—the applicant is not "compensated" for the PTO's administrative delay, he is benefitted by it.

But if subsection (B) had been intended to guarantee a 17-year patent term and *no more*, it could easily have been written that way. It is true that the legislative

context—as distinct from the legislative history—suggests that Congress may have intended to use subsection (B) to guarantee the 17-year term provided before GATT. But it chose to write a "[g]uarantee of no more than 3-year application pendency," 35 U.S.C. § 154(b)(1)(B), not merely a guarantee of 17 effective years of patent term, and do so using language separating that guarantee from a different promise of prompt administration in subsection (A). The PTO's efforts to prevent windfall extensions may be reasonable—they may even be consistent with Congress's intent—but its interpretation must square with Congress's words. If the outcome commanded by that text is an unintended result, the problem is for Congress to remedy, not the agency.



Norman A. THOMAS, Plaintiff,

v.

DISTRICT OF COLUMBIA  
GOVERNMENT, et al.,  
Defendants.

Civil Action No. 07-1286(HHK).

United States District Court,  
District of Columbia.

Sept. 30, 2008.

**Background:** Alien brought action seeking to have records of time he spent in state and District of Columbia custody corrected in order to be in better position to avoid order of removal. District and Department of Homeland Security moved to dismiss.